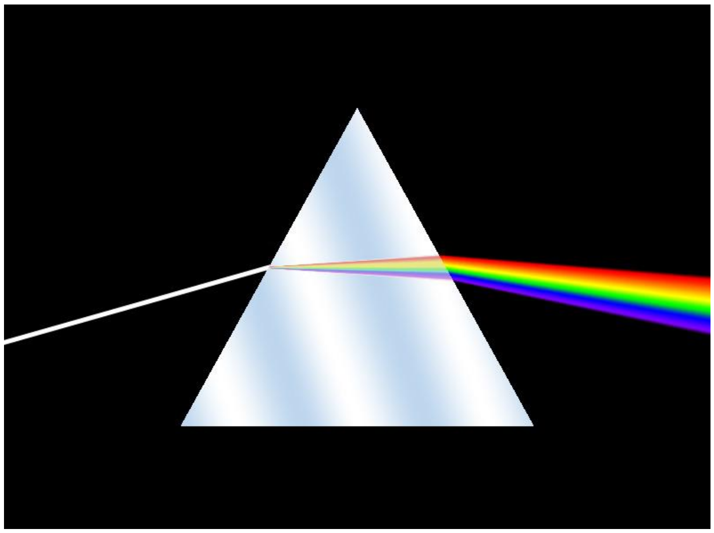
Washington State Patrol



Crime Laboratory Division

WASHINGTON STATE PATROL

MATERIALS ANALYSIS TECHNICAL PROCEDURES
CRIME LABORATORY DIVISION

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Contents

PART ONE: INTRODUCTION

1 OVERVIEW

The Crime Laboratory Division's (CLD) Materials Analysis section goal is to provide quality evidence assessment, identification, comparison, and reconstruction associated with forensic investigations. A Materials Analysis scientist will examine evidence in an attempt to:

- Place an individual at the scene of a crime and/or indicate that two or more people or things may have been in contact with each other.
- Identify that a certain material may have originated from a known or larger item.
- Demonstrate that two or more items were, at some time, one piece.
- Demonstrate that two or more materials may have the same chemical composition.
- Characterize and identify an unknown material.
- Help to determine the source of a material.
- Investigate a material's production/fabrication methods, manufacturer quality control and techniques (e.g., fibers, plastic bags) and evaluate the probative value of the material.

The Materials Analysis Technical Procedures Manual describes the methodology, techniques, and procedures to be used for the examination and analysis of evidence by all forensic scientists working in the section. It also provides requirements, suggests analytical routes and general information to assist the scientist in completing their analyses. No procedures manual can completely cover the myriad of situations and evidence types that forensic scientists may face; rather, it is a statement of methods and guidelines used by the WSP Crime Laboratory System. There may be valid reasons for using different procedures and/or techniques in situations which appear to be the same. The technical procedures manual is not meant to be a substitute for experience. The experienced forensic scientist is also expected to evaluate alternative procedures through specific outside agency workshops, identification manuals, references and pertinent literature.

The analysis of evidence seen in the Materials Analysis section can be accomplished by a variety of methods. Non-destructive tests should be performed first if practical. Limited sample size, the possibility of future analyses, and other limitations should be considered before destructive tests are performed. The integrity of the evidence is of utmost importance and evidence handling will be conducted in accordance with the Quality Operations Manual (QOM), Evidence Management.

It is desirable to have procedures in place for accomplishing any analysis, and if feasible, to allow the forensic scientist a choice of the best analytical procedure(s), taking into consideration sample variability, personal skills, and equipment availability. The scientist will use these procedures as per the QOM, Assuring the Quality of Test Results.

Creation and validation of new methods, procedures, or modification of current procedures, increases the experience, knowledge, and skill of a forensic scientist. This Technical Procedures Manual is meant to be a dynamic tool which will be reviewed yearly as per the QOM, Document Control Policy and Procedures.

After review of the Request for Laboratory Examination form, it may be necessary to contact the detective/agency to obtain pertinent case information and specific information about the submitted evidence. After this review and discussion with the detective/agency, the forensic scientist will decide on the best case approach and evidence prioritizations.
Different forensic disciplines may be called upon to examine the same item of evidence. The order in which the examinations will be conducted needs to be resolved on a case-by-case basis. The order of examinations should be selected and conducted so as to preserve the most transient evidence and provide the greatest discrimination and most valuable information.

Examiners must be aware or make the submitting agency aware of the effects that some disciplines' processing and examinations may have upon other specific examination requests.

Scientists shall evaluate the characteristics of unknown items prior to comparison to known items wherever possible. However, it is often necessary to determine the range of characteristics of known materials before a search for questioned samples can be conducted. It will be at the discretion of the scientist how best to proceed.

2 REVIEW OF THE REQUEST

During the review of the request not only should analytical capabilities be considered but also what sub-discipline the request is categorized as and which analysts are best suited for the nature of the request. Some requests may cross sub-disciplines which may require the generation of multiple requests to answer the questions being asked by the customer. Sub-discipline assignment will dictate which procedures to follow and conclusions that can be made. Upon physical inspection of the evidence it may be necessary to recategorize and/or generate additional requests.

2.1 GENERAL CHEMICAL ANALYSIS MATERIALS

Requests for analysis of unknown materials comprise a large variety of substances in a variety of states, conditions, and matrices. In order to provide a consistent approach to case acceptance, Materials Analysis supervisors need to follow a prescribed process for evaluating requests for appropriateness, in terms of what the laboratory is capable of reasonably analyzing within the capabilities and constraints of the scientists, equipment, and methods.

Types of samples that may be submitted can include, but aren't limited to:

- Determine the identity of an unknown material, which may be a solid, liquid, or residue
- Compare some unknown material to a known material with known properties
- Determine if a submitted material was contaminated with something (most often with an intent to cause harm)

Requests can be further categorized based on the type of substances involved, to include:

- Non-volatile organic solid or liquid material
- Volatile organic material
- Inorganic crystalline material
- Residues of OC spray or bank dye
- Residues of lubricants, whether water-based or organic-based
- Food or commercial product contamination

Note that the above are not all-inclusive of what may be submitted as an unknown substance request.

Upon receiving an RFLE or other inquiry (telephone call or email) regarding a request for an unknown substance analysis, the supervisor must ascertain, by communication with the customer, the pertinent details of the request as follows:

1. Does the customer suspect a specific target compound or class of compounds?
    - If yes, is the substance within the capabilities of the CLD? Questions 2 and 3 may still apply. Consider consulting with the other MA supervisors and/or technical leads to discuss.
    - If no, go to Question 2.
2. Is a comparison to some known material being requested?
    - If yes, is the known material also being submitted with the unknown? If so, the request is ok to accept.
    - If no, go to Question 3.
3. Is the matrix simple?

- If yes (e.g. a homogenous sample, or a simple mixture where the matrix material does not interfere with extraction and analysis), the request is ok to accept.
- A matrix will be considered complex if it can interfere with analysis, such as proteins and lipids that can bind to the target analyte. Does the CLD have the capability, expertise, and equipment to perform the requested analysis? Consult with the other MA supervisors and/or technical leads to determine whether to accept the request.

4. Is the material or matrix in which the suspected material contained in perishable? If so, how was the evidence stored prior to submission to the laboratory? Is there a high probability that spoilage has occurred, and could spoilage interfere with the analysis? In cases where the evidence was not packaged or stored appropriately to preserve the evidence, the case should not be accepted.

The decision to accept or reject the request will be based on the answers to the above questions, and discussion between supervisors and/or technical leads. When requests are accepted for analysis, the customer should be made aware that the crime lab may not be able to provide an answer depending on what the material is and any limitations of instrumentation and detection limits.

3 NOTE TAKING

Note taking for any examination provides a written document of what was received, observed and collected. Equipment and reagents (including mounting media) that are used during examinations will be noted in the case notes and appropriate instrument parameters listed. Refer to the QOM, Case Management additional information regarding note taking documentation.

If an observation, data, or a test result is rejected, the reason, the identity of the individual(s) taking the action and the date shall be recorded in the technical record.

Note taking can be handwritten on paper or done with the use of a form or worksheet. Notes may be taken electronically or in LIMS provided a hard copy is maintained in the case file. Refer to the QOM, Document Control Policy and Procedures for additional information regarding forms and worksheets.

4 TOOLS, SUPPLIES, INSTRUMENTS, DATABASES AND REFERENCE COLLECTIONS

A wide variety of tools, supplies, instruments, databases and reference collections are necessary for the diverse categories of testing performed in the Materials Analysis section. Each Materials Analysis section should be stocked with basic laboratory tools and supplies. The specific tools, supplies and instruments will be determined by the types of examinations performed in each lab.

4.1 CHEMICALS AND REAGENTS

Chemicals and reagents must be of the appropriate grade for the test performed. For most test procedures, reagent grade chemicals are sufficient unless specifically stated otherwise in the individual sections of this manual.

Reagents prepared in the laboratory shall be labeled with, at minimum, the identity of the reagent, the date of preparation or batch/lot number, and storage conditions if other than ambient. Records shall be maintained identifying the preparer of the reagent and the results of the reagent check. Reagent checks are confirmation of the reliability of the reagent after initial preparation and are performed each month for those reagents not intended for one time use. Chemical screening tests which are not considered a Seized Drugs Analysis Category 1 or Category 2 test will be reliability checked when prepared and as appropriate. Reagent reliability checks which consist of negative controls and positive controls using reference materials will be documented in a reagent logbook. One time use reagents may be documented in a reagent logbook or in the case notes. Gaps between checks are not a concern during periods of inactivity so long as the performance is checked prior to subsequent use. Written formulations for all chemical reagents seized drug color screening tests are listed in Appendix A.

Chemicals, including but not limited to, solvents and dilute acids and bases, are not considered reagents, and therefore do not require reliability checks. Purchased chemical containers must be dated and initialed when received and when first opened. All containers of chemicals that are prepared in the laboratory (e.g., dilute acids or bases) will be labeled with the identity of the contents and the date of preparation or batch/lot number. Personal use containers of chemicals not prepared in the laboratory (e.g., personal use containers of solvents) will be labeled with the appropriate name of the chemical.

4.2 DATABASES

- Footwear and tire databases and manufacturer website information
- LIMSRef Drug Reference database
- Paint Data Query (PDQ)
- Pharmaceutical imprint databases and reference materials
- Shoeprint Image Capture and Retrieval (SICAR)

4.3 REFERENCE COLLECTIONS

- Animal hair
- Botanicals
- Drug
- Explosives
- Fibers
- Human hair
- Ignitable liquids
- Mineral sets

- Paint-chip books
- Polymer set
- Vehicle lamps

5 TECHNICAL REVIEW

Technical review will be conducted on all cases as specified in the QOM Review of Casework – Technical Review.

Materials analysis utilizes three different technical review checklists depending upon the type of case being reviewed. Changes made to the technical records as a result of technical review will be tracked as follows:

- The “Yes” or “No” boxes for “Changes were made to technical records due to the technical review process” will be marked.
- Administrative changes to technical records (including the draft report) will be marked with “CIR” to indicate if the change was due to a Change In Review. Such changes include, but are not limited to, corrections to case numbers, dates, initials, page numbers, overwrites, or misspellings. No additional notations are required on the technical review checklist.
- Analytical changes or additional work conducted due to technical review will be specified in the notes section of the technical review checklist.

PART TWO: INSTRUMENTATION & TECHNIQUES

6 BALANCES

6.1 INTRODUCTION

Accurately determining the weight of evidence may be an important step in the course of analysis. Several different models of balances are used throughout the materials analysis functional area. This procedure is intended to be a general guide for the use of balances.

6.2 APPARATUS AND EQUIPMENT

- Electronic balance
- Small paint brush or similar brush
- Weighing vessels
- Certified 40 gram and 1 pound check (working) standards
- Balance manufacturer's operating manuals, if available

6.3 PROCEDURE

The function of a balance is greatly influenced by its location. Balances should be placed on a bench or table top that is level and does not transmit excessive vibration. The level bubble indicator, or other level indicator, should be used to determine if the balance is level. If the balance is not level, the feet should be adjusted until the balance is leveled. Balances should not be placed in areas that are subjected to drafts, electrostatic charges or magnetic fields. Balances should be left connected to the power supply and left on continuously so that a thermal equilibrium is established.

A weighing vessel will always be used with the balance and should be tared/zeroed prior to weighing the sample. The sample should be placed as close to the center of the pan as possible and should not extend off of the balance pan. If the balance is equipped with a draft shield, this should be closed prior to taking a reading. Once the material being weighed is placed on the balance, the reading will be taken when the mass display has stabilized. The balance used and the determined weight of the material will be recorded in the notes. Dynamic and static weighing are both considered two weighing events for the purposes of measurement uncertainty. Recording in the notes which weighing process was employed is optional.

Dynamic weighing is a process in which the weighing vessel is tared/zeroed and the material is placed directly on/in the weighing vessel.

Static weighing is a process in which the weighing vessel is tared/zeroed, the vessel is removed, the material is placed on/in the vessel, the vessel is returned to the balance.

Glassine paper or aluminum weighing vessels are preferable to polypropylene weighing vessels due to the significance of static in the weighing process.

6.4 QUALITY ASSURANCE

6.4.1 CALIBRATION

New balances will be calibrated by an external calibration service following installation. Weighing of surrogate samples to establish measurement uncertainty as described in the Measurement Uncertainty chapter of this manual will commence following external calibration of the balance.

Balances and mass standards will be calibrated by an external calibration service at least once a calendar year. The calibration certificates issued will contain the measurement results, including the measurement uncertainty and/or a statement of compliance with an identified metrological specification. Following calibration the balance and mass standards and their associated calibration certificates will be assessed before the balance and mass standards are placed back in service. Documentation of this assessment is done via the Calibration Check form which will be maintained in either electronic or paper format.

6.4.2 MAINTENANCE

A brush should be used to sweep away solids from the balance pan. If cleaning is required, a soft cloth dampened with water and a mild detergent can be used. Caution should be taken so liquids do not enter the balance. Harsh chemicals should not be used to clean the balance.

6.4.3 PERFORMANCE VERIFICATION

Each balance used for weighing seized drugs will be checked monthly
using the calibrated forty gram and one pound mass standards, as appropriate for the balance capacity. A weighing vessel will be tared/zeroed prior to placing the check standard on the balance. Cotton or other appropriate cloth or leather gloves will be worn when handling the check standard to avoid thermal conduction and contamination from the skin. The check standard must be equilibrated to the room temperature of the balance for a minimum of two hours prior to weighing. The determined weight of the check standard, date, and initials of the scientist will be recorded in a balance log.

When not in use, the mass standards will be stored in the padded box provided by the manufacturer. If the weight is dropped or becomes damaged, it will be taken out of service and recalibrated by an approved calibration company. This will be documented in the balance log book or electronic record.

Balances used for weighing seized drugs will be monitored monthly to determine if they are weighing within an acceptable range. A warning limit of two sigma (2σ) and a control limit of three sigma (3σ) will be established. If two consecutive measurement attempts are outside of the control limit, the balance will be taken out of service for evaluation. Maintenance and/or calibration may be required prior to returning the balance to service. This will be documented in the balance log book.

If a balance is taken out of service when two measurement attempts are outside of the acceptable range, the balance may be recalibrated using the manufacturer’s recommended external calibration procedure. A calibration procedure specific to the balance must be written and approved by the Materials Analysis-Chemistry Technical Lead and Quality Assurance Manager prior to the performance of the external calibration. Following the external calibration, linearity will be checked at a minimum of two additional points using calibrated mass standards, one of which may be the certified forty gram mass standard. The uncertainty associated with the mass standards used for the external calibration will be incorporated in the Measurement Uncertainty calculations for the balance.

6.5 **SAFETY**

As with all electronic equipment, caution should be taken to avoid electrical shock if the balance is exposed to liquids.

7 CAPILLARY ELECTROPHORESIS (CE)

7.1 INTRODUCTION

Capillary Electrophoresis (CE) is an instrumental separation technique for separating components of a mixture in a liquid based on analyte size, charge, and interactions with the liquid phase. Separations are carried out in capillary columns filled with a conductive liquid phase (run buffer or background electrolyte) and subjected to an electric field. Separations occur based on differences in analyte size and charge, or on analyte interactions with additives in the background electrolyte. The application of a high voltage to the capillary column generates electroösmotic flow in the capillary, resulting in a bulk flow of liquid toward one end of the capillary. This allows for the detection of separated analytes using various detection methods.

7.2 ADVANTAGES AND LIMITATIONS

Capillary electrophoresis is a separation method that generates high-resolution separations in a short analysis time. It is particularly well suited for thermally labile, non-volatile and water-soluble analytes. Ionic species are easily separated; however, uncharged analytes can also be separated. CE uses picogram or smaller quantities of materials making it an excellent method for limited sample sizes. Very small volumes of liquids and virtually no hazardous solvents are used. Concentration detection limits are limited; however, since CE analysis utilizes nanoliter or smaller injection volumes, the mass detection limits are excellent. The technique is most frequently used with either UV or fluorescence detection such that absolute structural identification of analytes is not obtained. UV detection provides limited structural information, thus CE with UV diode array detection (DAD) is a Category 2 technique. Rigorous migration time reproducibility for some methods is not possible; however, the use of internal standards and relative migration times (as indexed with respect to the internal standards) can be used.

Libraries can be created that may incorporate any combination of migration time data, spectral data, and quantitative data.

7.3 APPARATUS AND EQUIPMENT

The CE unit must have a means for introducing samples into an analytical column, analytical column temperature control, run buffer reservoirs, a high voltage power supply, electrodes, a safety interlock cabinet and a detection system. Other detection systems are commercially available including fluorescence, conductivity, and mass spectrometry detectors.

An autosampler designed to run sequential samples without an operator present is helpful, but not necessary for CE analysis.

CE grade solvents or better should be used for buffer preparation, sample extraction, and sample dilution. Other commercially available CE reagents may also be used.

Run buffers (background electrolytes) may be prepared in-house as needed or purchased from commercial vendors. Buffers are typically designed for a specific application or class of analytes.

Analytical columns are typically fused silica capillaries (ranging in size from 10 to 100 microns inside diameter and approximately 0.1 to 1.5 meters in length); however, special application capillaries with interior coatings or packing materials are available.

Various disposable plastic and glass lined capped vials are used for samples and run buffers.

7.4 PROCEDURE

Sample preparation is typically done by diluting or extracting a sample with CE grade water.

Samples may be introduced using various injection methods; such as, pressure injection or electrokinetic injection.

Separation methods are selected that are appropriate for the analytes of interest. For example, cation, anion, chiral, or neutral species methods have been designed to separate specific species.

Analytical columns appropriate for the selected method are used. Column tips should be protected to prevent chipping and the columns must not be allowed to dry out. It is typically best to store the ends of the column in CE water while not in use. A single analytical column may be used for more than one method; however, this often necessitates a lengthy run buffer equilibration time before the new method is ready for use. It is usually more expedient to have separate columns for each analytical method.

When changing from one method to another, it is sometimes (but not always) necessary to clean the electrodes and sample introduction device.

Detection is accomplished with the UV DAD. Spectral information from 190 to 600 nm may be collected. Each method is designed to monitor the separation at a wavelength appropriate to the analytes of interest.

Vial positions must be verified and documented.

Results will be recorded in the case notes and data included in the case file. The case file will also contain the following information for each sample CE run:

- Unique Identifier of the instrument used (unless the laboratory only has one instrument and that instruments identification is documented in the laboratory's equipment list.)
- The name of the method used
- The date of the run
- Sample name
- Buffer used
- Column length and inside diameter
- Column temperature
- Column preconditioning steps, if applicable
- Injection method and conditions
- Run voltage
- Detector monitoring wavelength

Other instrument conditions may be recorded in a logbook or other storage location as long as they are traceable and routine to the laboratory. If non-routine conditions are used, these will be recorded in the case file.

7.5 INTERPRETATION

Analytical reference materials appropriate to the method being used (and/or the analytes being examined) must be run in order to evaluate sample data. Absolute migration times in CE tend to be variable, so a comparison to appropriate internal standard(s) must be made in order to properly evaluate the data. Samples may be run with a non-interfering internal standard(s) and compare to migration times relative to reference materials run with the same internal standard(s). The sample may also be run and then the components confirmed by a second run where internal standards are added. Migration times relative to the reference materials should agree at better than ± 0.1 minute.

Data interpretation must be done taking into account any sampling bias resulting from the injection method.

When spectral data is used for analyte confirmation, the unknown analyte must be compared to the reference material both for relative migration time and spectral concurrence.

Since the run buffers are liquids at specific pH values, care should be taken in considering the effect of pH on the analytes of interest.

7.6 **QUALITY ASSURANCE**

7.6.1 CALIBRATION

The deuterium lamp is the only system that can be calibrated on the CE. The frequency of calibration is determined by the frequency of use of the instrument. Calibration is done using an empty red alignment interface and performing the DAD test and monitoring lamp count. This calibration must be done once a month, following any instrument maintenance, or as needed. A gap longer than a month between calibrations is not a concern during periods of inactivity (e.g., holidays) so long as the calibration is checked prior to subsequent use.

The CE DAD calibration records are kept in a logbook or on the system computer, which may be viewed under DAD test results in the software.

7.6.2 MAINTENANCE

The CE system is maintained by following the manufacturer recommendations. Three systems are identified for routine inspection/maintenance: 1) the injection system, 2) the replenishment system, 3) and the detection system. The injection system involves the cleaning of electrodes and prepunches. The replenishment system involves cleaning of reservoirs, electrode, o-rings and inlet frits. The detection system involves the optical alignment interface, capillary windows, and the deuterium UV lamp.

Maintenance on the CE system should be done according to the manufacturer’s recommendations as needed. The Agilent manual recommends approximately weekly checks, however, if the instrument is used only infrequently, it does not warrant as frequent calibration of the lamp or cleaning of the electrodes. System failures in these areas are obvious based on the inability to collect data if one of these systems is malfunctioning. Variations from published methods as far as capillary length, inside diameter, detection window path length, run voltage, injection time, and injection method may be made without revalidation as long as reference materials are run to verify system suitability.

7.6.3 PERFORMANCE VERIFICATION

Individual analysis methods also need to be performance checked using reference materials. This must be done at least once each day when the method is in use and a record of the performance check must be included in the case file. An appropriate preparation blank must be run to performance check the extraction/dilution solvent(s) to demonstrate that they are free from interfering substances. This performance check is conducted prior to running a case sample. CE (or comparable) grade solvents that pass the performance check may be used even if they have exceeded the manufacturer’s expiration date.

Upon installation of a new instrument, the service engineers will run a series of samples to ensure the instrument is functioning appropriately. Prior to use in case work, a series of reference materials, blanks and case type samples will be evaluated on the instrument. The service engineer evaluation data and the series of reference materials and samples will be filed with the maintenance log book.

7.7 SAFETY

A high voltage power supply is required to drive the separation in the CE. A safety interlock is in place to shield the user from the high voltage. This safety mechanism should not be tampered with.

Exposure to high powered UV can cause blindness and thermal burns. The interior top cover in the detector chamber should not be opened while the UV lamp is turned on.

The scientist should be aware of any hazards associated with chemicals used as buffers or sample preparation liquids. Appropriate Safety Data Sheets (SDS) should be on file.

8 GAS CHROMATOGRAPHY-FLAME IONIZATION DETECTION (GC/FID)

8.1 INTRODUCTION

Gas chromatography (GC) is an instrumental separation technique based on differences in the interactions of analytes between a stationary liquid phase coating on a solid support, and a mobile carrier gas.

8.2 ADVANTAGES AND LIMITATIONS

Gas chromatograph-flame ionization detection (GC-FID) systems are used in the characterization of unknowns based on retention time and column selectivity. GC can be used to evaluate patterns or individual peaks. GC is regarded as a Category 2 test for seized drug analysis.

Not all compounds lend themselves to GC analysis due to poor volatility or thermal degradation. Derivatization may be useful to improve the chromatography of such a compound, and may also give additional data for characterization and identification.

Commercial instrumental library searches are not possible for GC-FID data. The use of published tables of relative retention times to a specified compound or listed Kovats indices can be a useful starting point but should be used with caution. Appropriate references must be run under the same conditions as the sample and the retention differences must be within acceptable limits.

8.3 APPARATUS AND EQUIPMENT

A gas chromatograph must have an injection port or means of introducing a sample to a chamber (oven) which houses a column capable of separating compounds of interest. For gas chromatography (GC), the sample is injected through a heated zone (to volatilize the sample) onto a column (usually fused silica with a liquid stationary phase coated or bonded to the inner wall) and eluted using a carrier gas (He, H_2, etc.) as the mobile phase. Separated components of the mixture are detected, as they elute, by flame ionization detection (FID).

An autosampler designed to run sequential samples without an operator present is helpful, but not necessary, for GC analysis. Disposable glass vials with caps, appropriate for the model of autosampler employed, may be used.

Various syringes (sized anywhere from 0.01 to 500 microliters or more depending on the sample) can be used to manually introduce a sample onto the column if no autosampler is present on the system.

Reagent grade solvents or better should be used for extractions and dilutions.

The column selected must be adequate for the method.

The selected carrier gas must be of sufficient purity to meet manufacturer's suggested specifications and must not interfere with the quality of the analysis.

8.4 PROCEDURE

Samples for GC analysis should be diluted or extracted with organic based solvents. Direct injections are acceptable with due caution. Such injections should be clear and free of solids, acids, bases, dissolved polymers and other high boiling point components. Extractions, filtration, and/or centrifugation help to eliminate unwanted components that may contaminate the inlet system or shorten the life of the column. Excessive water can be very damaging to a capillary column. Extreme care must be taken to ensure that the correct layer of a sample extraction is placed in the GC vial for analysis. Strong acids and bases will destroy a GC column by stripping off the column's stationary phase.

Samples can be injected manually or via an autosampler. Appropriate blanks will be run prior to each sample. The syringe must be rinsed with an adequate solvent between samples and a preparatory blank run. Syringes should be checked periodically for wear and blockage and replaced if necessary.

A capillary column and method which are capable of resolving the substance in question from all closely eluted compounds needs to be evaluated prior to analysis of the target compound of interest (e.g., for methamphetamine analysis, check with closely eluting compounds - phentermine, ephedrine, etc.). A change in the column or method, including temperature, flow, pressure, and/or carrier gas, requires an evaluation of the method to be performed.

Results will be recorded in the case notes.

Vial positions must be verified and documented.

The case file will also contain the following information for each sample GC-FID run:

- Unique Identifier of the instrument used (unless the laboratory only has one instrument and that instruments identification is documented in the laboratory's equipment list.)
- The name of the method used
- The temperature program used by that method
- The column stationary phase and physical dimensions (internal diameter, film thickness, length)
- Carrier gas
- Injection volume
- Split ratio

Other instrument conditions may be recorded in a logbook or other storage location as long as they are traceable and routine to the laboratory. If non-routine conditions are used, these will be recorded in the case file.

Information specific for the use of the GC-FID for quantitation is addressed in the Marijuana chapter of this manual.

8.5 INTERPRETATION

An analytical reference material for each substance to be identified or other compound reported needs to be run in order to evaluate sample data. Chromatographic quality is evaluated by peak symmetry and baseline resolution. In some cases, less than ideal chromatography may be accepted and may still provide adequate quality data.

The retention time of the sample should agree within ± 2%, or ± 0.03 minutes (whichever is greater) of the retention time of the reference material to be considered acceptable. This is calculated by the retention time difference between sample and reference material divided by the retention time of the reference. When evaluating the retention times, consider method parameters, concentration, peak shape and sample preparation conditions. For example, a large difference in the concentration of the reference material and the sample will commonly lead to differences in retention time. This can be remedied by diluting or concentrating the reference material or the sample and re-injecting to achieve a better comparison between sample and reference retention times for determining the suitability of the data. Any variance greater than the range specified should be further evaluated: it will be considered acceptable with adequate scientific justification and concurrence of the reviewer. The justification must be indicated clearly in the case notes.

8.6 QUALITY ASSURANCE

8.6.1 CALIBRATION

GC-FID systems do not require calibration.

8.6.2 MAINTENANCE

Mechanical maintenance of GC systems is conducted per manufacturer's recommendations or as needed, as is replenishment of carrier gases.

8.6.3 PERFORMANCE VERIFICATION

Retention data for chromatography must be determined for each individual instrument by running appropriate reference materials. Gas chromatographic retention times for reference materials should be obtained periodically, following a change in the length of the chromatographic column, or as needed.

The instrument performance will be evaluated weekly by running a drug test mix consisting of methamphetamine, phentermine, cocaine, and one compound eluting in the final third of the test mix run (such as alprazolam, noscapine, or oxycodone), but may also include additional compounds. Peak shapes, intensities and ratios are evaluated for consistency with past runs of the known test mix (based on the scientist's training and experience). Any significant change in chromatography or GC retention time (+/- 1% or +/-0.015 min., whichever is greater, of the previously run drug test mix) will be addressed before the instrument is used in case work. A printout of the test mix will be initialed by the scientist and will be maintained as part of the instrument records. Gaps between checks are not a concern during periods of inactivity (e.g., holidays), as long as the performance is checked prior to subsequent use.

New instruments or instruments transferred from one lab to another will be verified by running a series of reference materials over at least a one week time period. The retention times of the reference materials will be evaluated for drift over the evaluation period.

8.7 SAFETY

All systems should be turned off and cooled prior to performing maintenance.

Cylinders containing compressed gases are under high pressure and are therefore potentially explosive. The cylinders must be stored and transported correctly, chained or strapped to a bench, wall or other approved device, and kept capped when not in use. Appropriate regulators must be used with each gas cylinder. If utilizing bottled hydrogen as the source of the GC carrier gas, extreme care must be taken to ensure there are no leaks or accumulation of hydrogen within or around the instrument. Leak detecting equipment should be used periodically to monitor valves and carrier gas lines for potential hydrogen leaks. Eliminate as many ignition sources as possible when utilizing hydrogen as a carrier gas. The installation of an automatic valve that will shut off hydrogen flow in the event of a power failure is recommended when bottled hydrogen is utilized.

9 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC/MS)

9.1 INTRODUCTION

GC/MS is an analytical instrument that combines the separation capabilities of gas chromatography (GC) with the sensitivity and selectivity of mass spectrometry (MS). MS is a highly sensitive and versatile instrumental technique for a wide variety of organic and inorganic compounds. When coupled with gas chromatography (GC), sample preparation can be simplified and complex mixtures are readily analyzed. Upon introducing a sample into the MS the sample is ionized and fragmented into charged ions. The resulting ions are then separated according to their mass to charge (m/z) ratio. This may be accomplished with a scanning quadrupole magnetic field, an ion trap, or another filtering technique. A detector and data system (computer) record the mass and quantity of ions as the spectrometer is scanning, resulting in the generation of a mass spectrum. The computer software is used to acquire data in a form which can be used by the scientist and which does not alter the fundamental and/or raw data. Retention times in the total ion chromatograph (TIC) and mass spectra (MS) from a sample may then be compared to those of reference materials for purposes of identification.

Pyrolysis, the controlled, reproducible thermal decomposition of a sample, allows the introduction of non-volatile polymeric materials into a gas chromatographic system by breaking bonds to create smaller, more volatile components. Polymeric materials will fragment predictably when subjected to the same pyrolysis conditions, allowing for comparison of an unknown material to a reference material. Pyrolysis of polymers often results in breakdown into component monomeric units, enabling identification of polymers based on GC-MS data of the break-down products. Evidentiary materials, such as paints, are often complex polymers with several major and minor monomer constituents, making direct identification based on GC-MS data impractical. Therefore, many pyrolysis GC-MS analyses in forensic science are comparative in nature.

9.2 ADVANTAGES AND LIMITATIONS

GC/MS is regarded as a Category 1 test for seized drug analysis. An advantage is that the systems can be automated to increase analytical time efficiency. Limitations include complete destruction of the analyzed portion of the sample and the fact that thermally labile and non-vaporizable samples are not readily analyzed. Mass spectra from closely related compounds and isomers may be so similar that conclusive identification is not possible.

Pyrolysis allows for the analysis of non-volatile polymeric materials in a gas chromatograph/mass spectrometer. An advantage of pyrolysis GC/MS that it is a sensitive technique that in many cases can detect minor differences that would otherwise be obscured by strong absorption bands in FTIR analysis. A limitation of pyrolysis is that it is a destructive technique, and should be used only when sufficient sample is available for further testing.

9.3 APPARATUS AND EQUIPMENT

Most of these systems utilize gas chromatography as a separation technique and quadrupole mass spectrometers as analyzers, and are capable of analyzing mass units between 10 and 650. All systems are equipped with computers and software. The pyrolyzer is controlled through a software interface, and also communicates with the GC-MS system so that GC runs are started together with a pyrolysis program.

An autosampler designed to run sequential samples without an operator present is helpful, but not necessary, for analysis. Disposable glass vials with caps and pyrolysis supplies, appropriate for the model of equipment employed, will be used.

Various syringes (sized anywhere from 0.01 to 500 (µL) or more depending on the sample) can be used to manually introduce a sample onto the column if no autosampler is present on the system.

Reagent grade solvents or better should be used for extractions and dilutions.

The column selected must be adequate for the method.

The selected carrier gas must be of sufficient purity to meet manufacturer's suggested specifications and must not interfere with the quality of the analysis. Carrier gas recommendations and information about the purity of the employed carrier gas will be documented in the equipment log book.

9.4 PROCEDURE

Refer to the GC/FID chapter for information related to GC. A sample may be introduced into the mass spectrometer by various means. Most frequently the MS is coupled to a GC equipped with a capillary column.

Samples can be injected manually or via an autosampler. Appropriate blanks will be run prior to each sample. The syringe must be rinsed with an adequate solvent between samples and a preparatory blank run prior to each sample to ensure that carryover does not occur. Syringes should be checked periodically for wear and blockage and replaced if necessary.

The pyrolysis unit has adjustable settings for the valve oven and heated transfer line isothermal zones, and programmable pyroprobe burn parameters for initial temperature, heating rate, and final temperature. The valve oven and heated transfer line should be set high enough to prevent condensation of pyrolysates. Pyrolysis heating parameters can be varied depending on the sample, but should be the same throughout a comparison of samples.

Samples to be pyrolyzed are held in place in a quartz tube using quartz wool and/or quartz filler rods. Care should be exercised to ensure that sample sizes are similar between samples to be compared. GC parameters such as carrier gas flow rates and oven temperature programs should be appropriate for the sample being run. Multiple runs should be performed to confirm reproducibility of the data, with a preparatory blank run immediately before the corresponding sample. Also, cleaning blanks will be run as needed.

A capillary column and method which are capable of resolving the substance in question with all closely related compounds needs to be evaluated prior to analysis of the target compound of interest (e.g., for methamphetamine analysis, check with closely eluting compounds - phentermine, ephedrine). A change in the column or method, including temperature, flow, pressure, and carrier gas, requires an evaluation be performed.

When background subtraction is performed, the case file will include the raw data mass spectrum, the mass spectrum being subtracted and the resultant mass spectrum.

Results will be recorded in the case notes.

Vial positions on the automatic liquid sampler and the quartz tube positions on the pyrolysis autosampler must be verified and documented.

The case file will also contain the following information for each sample GC-MS run:

- Unique identifier of the equipment used (unless the laboratory only has one instrument and that instrument's identification is documented on the laboratory's equipment list.)
- The name of the method used

- The temperature program used by that method
- Date and time
- The column stationary phase and physical dimensions (internal diameter, film thickness, length)
- Carrier gas
- Injection volume
- Split ratio

Other instrument conditions may be recorded in a logbook or other storage location as long as they are traceable and routine to the laboratory. If non-routine conditions are used, those will also be recorded in the case file.

9.5 INTERPRETATION

Once the sample has been run, mass spectral data must be compared to mass spectra of reference materials run under similar conditions for the purposes of compound identification. Reference libraries are useful for preliminary evaluation of unknown compounds. A comparison of the mass spectra, which includes comparison of relative peak heights, of reference material and sample must be made and any peak anomalies evaluated for their significance. Samples that exhibit many extra peaks in the TIC and spurious ions in the mass spectra may be mixtures of co-eluting compounds. In such cases, it may be necessary to perform additional analytical work in order to obtain mass spectra for non-co-eluting compounds. For substances that are not reported, mass spectra may be compared to reputable literature spectra.

Pyrolysis can be used to identify unknown polymeric materials by comparing pyrogram patterns and mass spectral data of the unknown material against reference materials. If the known and unknown pyrograms have the same peaks, with the similar peak intensities, then the samples can be associated as possibly having a common origin. Careful consideration of small differences in minor peaks and/or peak ratios should be made to ascertain if real differences in the samples exist or if the differences can be attributed to differences in sample preparation or contamination. Comparison of the mass spectra of major peaks can also be used to verify the chemical makeup of pyrolysates. Specifics on interpretation of pyrograms and associated mass spectral data are detailed in the sub-discipline technical procedures.

Refer to the GC/FID section for interpretation of peaks in the total ion chromatogram.

Each sample TIC/mass spectrum that is used to confirm a reported substance will be maintained in the case file along with a copy of the reference material TIC/mass spectrum. Data from the reference material will include the name of the reference material and lot number or lab ID number of the reference material used. This information allows for the reference material to be traced back to its source that includes all of the supporting documentation of that reference.

See the Fire Debris Analysis chapter of this manual for additional details pertinent to ignitable liquid analysis.

9.6 QUALITY ASSURANCE

9.6.1 CALIBRATION

The mass spectrometer is tuned by either of two means, manually or by an instrumental (computer) program which adjusts the MS operation parameters to achieve certain predefined performance criteria that optimize peak shape and position. The tuning typically utilizes perfluorotributylamine (PFTBA), a stable compound which produces ion fragments throughout the mass range for the spectrometer, to properly tune the mass spectrometer's electronics. These ions are used to calibrate and normalize the mass spectrometer's operation. This tune procedure is done only as needed. A tune must be performed after replacing the column, maintenance of the mass spectrometer, a system shutdown, or a parameter out of manufacturers' recommended range. A tune must also be completed when there are changes to the ion source temperature, or MS transfer line temperature. Remember to always keep the GC oven temperature, ion source temperature, MS transfer line temperature, and column flow rate constant when performing the tune. A complete discussion on tuning can be found in the manufacturers' operations manuals and/or software.

9.6.2 MAINTENANCE

Routine maintenance is performed as specified by the equipment operations manual/software at appropriate intervals and documented in the instrument log book. Deviations from the manufacturer's intervals are acceptable if, for example, less frequent use as long as justification is noted in the instrument logbook.

9.6.3 PERFORMANCE VERIFICATION

The instrument tune is checked each day an analytical sequence is started by performing either a "Tune Evaluation" or a "Generate Report" (or other equivalent report to evaluate the tune parameters). The report will be maintained. All parameters on the System Verification Report for the Tune Evaluation must say OK to be considered satisfactory for use. If Generate Report (or other equivalent report) is used, the report will be evaluated by a scientist to determine that the following conditions are met:

Isotopic Ratios	
70/69	0.38 – 1.82
220/219	2.90 – 5.90
503/502	8.20 – 13.80

Mass Assignments
+/- 0.1 amu

Peak Width (at half height)
0.40 – 0.70 amu

Air and Water Check	
Ratio of 18/69 (water)	<20%
Ratio of 28/69 (nitrogen)	<10%

The equipment performance will be evaluated weekly by running a known test mix such as, but not limited to, the following: drug test mix, polypropylene reference material, or ASTM E1618 test mix. The drug test mix will be run on instruments used for the analysis of seized drugs and will consist of methamphetamine, phentermine, cocaine, and one compound eluting in the final third of the test mix run (such as alprazolam, noscapine, or oxycodone), but may also include additional compounds. Peak shapes, intensities and ratios are evaluated for consistency with past runs of the known test mix (based on the scientist's training and experience). Any significant change in chromatography, GC retention time (+/- 1% or +/-0.015 min., whichever is greater, of the previously run drug test mix or ASTM E1618 test mix run), or fragmentation pattern that causes concern with instrument performance will be addressed before the instrument is used in case work. A printout of the test mix TIC and at least one mass spectrum of one of the compounds will be initialed by the scientist and will be maintained as part of the instrument records. Gaps between checks are not a concern during periods of inactivity (e.g., holidays), as long as the performance is checked prior to subsequent use.

Additional criteria, guidelines, or desirables may be used for the tunes of individual instruments. Refer to the instrument manual/software for additional details on tune evaluation.

New instruments or instruments transferred from one lab to another will be verified by running a series of reference materials over at least a one week time period. The retention times of the reference materials will be evaluated for drift over the evaluation period. Spectral data of the reference materials will be compared to literature and documentation of these comparisons will be kept with the verification paper work.

Refer to the GC-FID chapter of this manual for more specific details on the gas chromatographic portion of this procedure.

9.7 **SAFETY**

All systems should be cooled prior to performing maintenance. When applicable, the instrument should be turned off and electronic components unplugged prior to performing specific maintenance functions. Additionally, pyrolysis system components (valve oven and heated transfer line) are hot and should be turned off and allowed to cool prior to performing maintenance.

Scientists should be aware that there are two sources of exhaust on the GC/MSD system: the foreline pump and the GC split vent. The foreline pump outputs gas removed from the vacuum manifold by the high vacuum pumps. The foreline pump exhaust will also contain traces of solvent and sample.

Caution should be exhibited when working with vacuum pumps. Waste oil should be treated as hazardous and should be handled and disposed of appropriately.

Cylinders containing compressed gases are under high pressure and are therefore potentially explosive. The cylinders must be stored and transported correctly, chained or strapped to a bench, wall or other approved device, and kept capped when not in use. Appropriate regulators must be used with each gas cylinder. If utilizing bottled hydrogen as the source of the GC carrier gas, extreme care should be taken to ensure there are no leaks or accumulation of hydrogen within the instrument. Leak detecting equipment should be used periodically to monitor valves and carrier gas lines for potential hydrogen leaks. Eliminate as many ignition sources as possible when utilizing hydrogen as a carrier gas. The installation of an automatic valve that will shut off hydrogen flow in the event of a power failure is recommended when bottled hydrogen is utilized.

10 GAS CHROMATOGRAPHY-FOURIER TRANSFORM INFRARED SPECTROSCOPY (GC/FT-IR)

10.1 INTRODUCTION

GC/FT-IR is a technique combining the separation capabilities of gas chromatography (GC) with the specificity of infrared spectroscopy. Analogous to GC/MS analysis, the GC effluent, comprised of various separated volatile components, is sequentially subjected to a spectrometric analysis. For GC/FT-IR this analysis is a measurement of the component absorptions in the mid-infrared region. This measurement occurs as the various separated components flow through a miniature vapor cell. The end of the GC column is attached to this vapor cell (commonly known as a light pipe), which consists of a 10 to 15 cm long metal tube having an inner diameter of 1 mm. The interior surface of the light pipe is coated with a layer of gold, which is both chemically inert and highly reflective. The analysis beam from the FT-IR interferometer is condensed and concentrated as it enters one end of the light pipe, where it then undergoes multiple reflections until it reaches the other end. The effective path length of the infrared analysis is thus greater than the actual length of the light pipe. The transfer lines to and from the light pipe are situated perpendicular to the light pipe, which has KBr windows on both ends, and this entire assembly has a “U” shaped configuration. The transfer line from GC column and the light pipe are both heated to prevent condensation of analytes.

The infrared absorptions of vapor samples are thus measured, and an algorithm using an integrated Gram Schmidt reconstruction is used to display the chromatogram. This reconstruction is based on interferograms, not spectra, and it provides a measure of the total infrared absorption occurring in the spectral region of interest. The Gram Schmidt chromatogram is thus analogous to the total ion chromatogram in a GC/MS analysis.

10.2 ADVANTAGES AND LIMITATIONS

A GC/FT-IR analysis is a Category 1 test for seized drug analysis. Along with structural data provided by infrared absorptions, a chromatographic retention time also provides additional characterizing information. Compared to an infrared analysis of a condensed phase (solid or liquid) sample, the main advantage of a GC/FT-IR analysis is the efficient separation of mixtures into individual components. Mixtures that are difficult to resolve using conventional partition or particle-picking isolation techniques may be readily analyzed, producing infrared spectra of individual components free of extraneous absorptions. Differences in infrared absorptions caused by polymorphism, hydration, and lack of crystallinity are also not issues when dealing with vapor phase spectra, and highly reproducible spectra are obtained. Unlike a mass spectral analysis, differentiation of isomers is much easier with infrared spectroscopy. Consequently, the ability of a GC analysis to separate isomers is less of a concern for a GC/FT-IR analysis compared to a GC/MS examination.

Similar to a GC/MS examination, a GC/FT-IR analysis is a destructive technique (in practice at least, although analytes are not actually destroyed), and only volatile constituents can be chromatographed. Thermally labile compounds may undergo decomposition and spectra of intact compounds may be greatly diminished in intensity or not present at all. Chromatographic separation efficiency is considerably less than that of a GC-FID or GC/MS analysis, and eluting peaks in the Gram Schmidt chromatograms usually display nonsymmetrical shapes (“bearding”) indicative of overloading. As noted, however, chromatographic separation ability is less of a concern, as even two compounds that co-elute will produce different absorption patterns. Because GC/FT-IR is a less sensitive technique than GC/MS, it is usually not helpful for dilute solutions. When splitless injections are required for GC/MS identification, these same samples are usually not concentrated enough to produce GC/FT-IR infrared spectra having sufficient signal to noise ratios for confirmation.

Compared to infrared spectra of organic compounds obtained in the crystalline state, GC/FT-IR spectra of these same compounds lack the details that result from numerous narrow absorptions that occur over a wide spectral range. GC/FT-IR infrared absorptions, which are un-resolved composites of a sequence of very closely-spaced vibrational-rotational lines, consist of relatively broad bands and usually lack fine structures. GC/FT-IR instruments also require the use of narrowband MCT detectors, and infrared data are normally collected at 8 cm^{-1} resolution to be able to collect as many scans as possible during the brief interval when analytes are passing through the light pipe. Although this lower spectral resolution normally has little bearing on these vapor spectra, the narrowband MCT has a more limited spectral range (with a low frequency cut-off near 700 cm^{-1}) than possible with most FT-IR spectrometer bench accessories.

10.3 APPARATUS AND EQUIPMENT

Compared to gas chromatographic analyses on both GC/FID and GC/MS instruments, the GC analysis for a GC/FT-IR system differs in several respects. Because infrared spectroscopy is inherently less sensitive than mass spectrometry, larger sample sizes are required, particularly when the ability to reduce noise through signal averaging is severely limited because of the “on-the-fly” nature of obtaining spectra of eluting vapors. Consequently, GC/FT-IR analyses normally require splitless GC injections, and wide bore capillary columns are used instead of the narrow bore ones used for GC-FID and GC/MS analyses. One important consideration for the choice of GC carrier gases is that they do not absorb in the mid-infrared region, but this is not a problem with either helium or hydrogen. However, the selected carrier gas must be of sufficient purity to meet manufacturer’s suggested specifications.

10.4 PROCEDURE

The GC procedures are essentially the same as those detailed in Gas Chromatography-Flame Ionization Detector chapter of this manual. As with GC-FID and GC/MS, extraction blanks will always be run prior to samples.

Before each run (blank or sample), the instrument acquires a background reference spectrum. All subsequent infrared data acquired during the run are ratioed to this background. Infrared spectra are obtained from Gram Schimdt chromatograms by selecting a region of the chromatogram over which individual spectra are to be co-added. The interferometer is scanning approximately three times per second, and as the chromatogram peaks of splitless injections are relatively broad, this typically involves co-adding several dozen individual spectra. In some cases, there will be residual carbon dioxide and water vapor absorptions in the sample, and a spectrum from a background region, chosen either before or after the sample elutes, is subtracted from the spectrum of the sample This may not always be necessary, and scientists must decide if this subtraction, which introduces additional noise into the final spectrum, is warranted.

At a minimum, case files should include the Gram Schmidt chromatogram labelled with retention times and the infrared spectrum of the analyte or analytes of interest. It is not necessary that spectra of every minor component be included in the case file. For spectral subtractions of backgrounds involving only carbon dioxide absorptions, minuend and subtrahend spectra do not have to be included in the case file, as long as the relevant elution intervals of these are indicated on the final difference spectrum. When spectral subtraction are used for removal of absorptions of a co-eluting component or components, however, the minuend and subtrahend spectra should be included in the case file, with the subtraction factor indicated on the final difference spectrum. A Gram Schmidt chromatogram and spectrum of the reference standard should also be included in the case file. In addition to case submission information, case files should include unique Identifiers of the GC and FT-IR instruments and the date and times of the analysis, along with:

Additional GC Information:
- The name of the GC method used
- The temperature program used by that method

- The column stationary phase and physical dimensions (internal diameter, film thickness, length)
- Carrier gas
- Injection volume
- Split ratio

Additional FT-IR Information:

- The number of background scans
- The number of sample scans
- Spectral resolution
- Sample gain
- The retention time interval used for the co-added spectrum of the sample
- The retention time interval used for the co-added spectrum of the background (if used)
- The subtraction factor (if relevant)

Vial positions must be verified and documented.

10.5 INTERPRETATION

Interpretation of GC and infrared data are discussed at length in the Gas Chromatography-Flame Ionization Detector and Infrared Spectroscopy chapters of this manual. Here, only issues specific to a GC/FT-IR analysis are discussed.

Peaks in the Gram Schmidt chromatogram for seized drugs and other types of evidence with single or few components will be evaluated for their significance and documented. The retention time of the sample should agree within ± 4%, or ± 0.3 minutes (whichever is greater) of the retention time of the reference material to be considered acceptable. This is calculated by the retention time difference between sample and reference material divided by whichever retention time is smaller. Any variance greater than the range specified must be further evaluated: it will be considered acceptable with adequate scientific justification, concurrence of the reviewer, and the justification indicated in the case notes.

When using GC/FT-IR infrared spectra for identification, a peak to peak comparison of a pure reference material run on the GC/FT-IR instrument and sample spectra should be made, and any significant differences must be noted on the spectrum. For cases where two components may be co-eluting, scientists should obtain infrared spectra of several different intervals throughout the Gram Schmidt chromatographic peak to determine if useable data can be extracted. Spectral subtraction results involving minuend and subtrahend spectra from different intervals of the peak should also be examined to determine if these produce analyte spectra free of extraneous absorptions.

10.6 QUALITY ASSURANCE

10.6.1 CALIBRATION

GC/FT-IR systems do not require calibration.

10.6.2 MAINTENANCE

Routine maintenance is performed as specified by the equipment operations manual/software at appropriate intervals and documented in the instrument log book. Deviations from the manufacturer's intervals are acceptable if, for example, less frequent use as long as justification is noted in the instrument logbook.

10.6.3 PERFORMANCE VERIFICATION

The performance of the GC/FT-IR system will be monitored as follows. A weekly drug test mix containing five compounds eluting over a wide range (which may include methamphetamine, phentermine, cocaine, diazepam, and zolpidem) will be run and evaluated for both GC retention times and infrared spectral data. The absorption frequencies of cocaine will be compared to those of previous runs, as a means to monitor the stability of the interferometer/narrowband MCT detector (which is separate from the detector used on the FT-IR spectrometer bench) system. The frequencies, in wavenumbers should be within 4 wavenumbers of those of previous weekly runs. If these criteria are not met, the bench should be realigned using the GC/FT-IR narrowband MCT detector. A printout of the test mix Gram Schmidt chromatogram and the infrared spectrum of each of the test mix compounds will be initialed by the scientist and will be maintained as part of the instrument records. Gaps between checks are not a concern during periods of inactivity, as long as the performance is checked prior to subsequent use.

10.7 SAFETY

GC safety precautions are described in the Gas Chromatography-Flame Ionization Detector chapter of this manual. The GC/FT-IR system uses a MCT detector, which must be cooled with liquid nitrogen. Appropriate safety precautions should be employed when filling the liquid nitrogen Dewar. Personal protective equipment including safety goggles, face shields, insulating gloves and long sleeves may be appropriate when handling liquid nitrogen.

11 GLASS REFRACTIVE INDEX MEASUREMENT (GRIM)

11.1 INTRODUCTION

Glass Refractive Index Measurement (GRIM) is both a tradename of the Foster + Freeman company and a generic term for semi-automated glass refractive index (RI) measurement system. For this manual, the abbreviation GRIM will be used in the generic sense. The GRIM uses a phase contrast microscope to magnify the image of glass particles that are immersed in a silicone oil at a fixed wavelength; monochromatic filters are used to obtain the fixed wavelength. The microscope is aligned to produce even illumination with maximum contrast between the glass particles and the surrounding oil. A video camera attached to the microscope relays to the software the contrast between the image of glass and the surrounding oil. The software then calculates the variance in pixel intensity for the region of interest. The temperature of the oil is changed via the hot stage and an electronic temperature controller until the glass particle is no longer visible in the immersion oil. The temperatures of a cooling cycle and a heating cycle corresponding to the minimum contrast (the point where the RI of the glass particle and the immersion oil are equal) are recorded. The average of these measurements corresponds to the match temperature. For both the heating cycle and the cooling cycle, the program calculates the edge count, which is the slope between the two points on the match temperature null plot and another point of the curve 0.2°C before the null point. The match temperature can be converted to RI by reference to a calibration curve for the immersion oil previously created from the match temperatures obtained on reference glasses of known RI.

11.2 ADVANTAGES AND LIMITATIONS

The GRIM can be used to determine the RI of glass to five decimal places, over a range of approximately 1.464 to 1.556. The measurement process is semi-automated. The instrument software also calculates the mean and standard deviation of multiple measurements of the same glass. Normally, glass fragments are crushed in preparing them for GRIM measurement, so the technique is not entirely nondestructive. Contaminants on the glass may interfere with the measurement, so glass fragments may require cleaning before the RI can be measured. Opaque and nearly opaque glasses may be difficult or impossible to measure with the GRIM, as the glass will always be visible in the silicone oil.

11.3 APPARATUS AND EQUIPMENT

The GRIM requires that the samples be mounted in a high stability silicone oil. The choice of the oil is dependent on the RI of the glass. A set of reference glasses of known RI is used for calibration of the instrument with each of these oils.

11.4 PROCEDURE

The microscope components will be aligned and optimized for maximum contrast. A Sodium D filter is placed between the light source and the sample. At the analyst’s discretion, other filters may be used to measure the refractive index at other wavelengths. The refractive index of the sample must fall within the range of the calibration curve to be used. Detailed instructions for the operation of the GRIM are found in the GRIM User Manual.

11.5 INTERPRETATION

The GRIM is designed for glass analysis. Therefore guidelines for interpretation of GRIM data are found in the glass section of this manual.

11.6 QUALITY ASSURANCE

The instrument will have a logbook to document, at a minimum, the days the instrument is used, calibrations, maintenance (excluding routine cleaning), the results of performance verifications, the specifications of each calibration curve (lot number of silicone oil, reference glasses used, and filter), damage, malfunctions, modifications, repairs, and when the instrument is placed out of service. Each of these points will include the relevant date(s) and person(s).

11.6.1 CALIBRATION

Calibrations should be performed using reference glasses across the range of the refractive indices for each combination of silicone oil and monochromatic filter. Silicone oils may degrade over time, especially if exposed to air and light. They should be stored appropriately to minimize these exposures. The following silicone immersion oils and reference glasses from Locke Scientific are preferred. The letter designation of the reference set of glasses will match the letter designation of the oil for any calibration performed.

Immersion Oils

Oil A	methylphenylpolysiloxane
Oil B	methylphenylpolysiloxane
Oil C	tetramethyltetraphenyltrisiloxane

Certified Reference Glasses

Set A	5 glasses	labeled A1-A5	n_D range 1.53990 – 1.55663
Set B	12 glasses	labeled B1-B12	n_D range 1.50187 – 1.52903
Set C	2 glasses	labeled C1-C2	n_D range 1.46409 – 1.48652

The GRIM will be calibrated at least once yearly at the Sodium D wavelength filter with the silicone B oil with at least 9 of the “B” reference glasses using the procedures outlined in the user manual. A straight line fit will be generated from the data points. Calibrations with the silicone A and C oils and the other wavelength filters will be done on an as-needed basis. Most types of glass encountered in casework fall within the working range for the B reference set.

The instrument will be recalibrated with a new lot of silicone oil prior to using that oil in casework. Calibration after changing the light source bulb is optional if the daily check (see Verification) using a reference glass are within tolerances.

11.6.2 MAINTENANCE

The GRIM is a robust instrument that requires minimal user maintenance and no manufacturer’s maintenance. User maintenance will be performed on an as needed basis and is limited to routine cleaning and replacement of the microscope lamp when it burns out. Routine cleaning will include keeping the microscope and optics clean. Exterior lens surfaces may be cleaned using lens cleaner, methanol, and/or other alcohols.

Outside service, change of lamp filaments, or any maintenance (excluding routing cleaning) to any part or accessory of the instrument must be recorded in the logbook.

11.6.3 PERFORMANCE VERIFICATION

The performance of the instrument using a specified calibration curve will be verified using a laboratory designated reference glass referred to as the verification glass (specific for that calibration curve). The calibration curve used will be specified by the oil and filter. The instrument will be successfully verified each day of casework use prior to the casework samples with a verification glass that has a refractive index that falls within the range of the calibration curve being used. If multiple calibration curves will be used that day, then each curve will be verified for that day. Successful verification is defined as the match temperature of the verification glass falls within ±0.2 degrees of the calculated temperature. The verification glass used will be different than the glasses used for generating the calibration curves.

11.7 **SAFETY**

Hazards associated with the silicone oils are found in the appropriate SDS.

See also the safety section in microscope chapter of this manual.

12 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

12.1 INTRODUCTION

High performance liquid chromatography (HPLC) is an instrumental analytical method that separates compounds using a combination of the appropriate stationary phase, mobile phase, column type, column temperature program, and mobile phase flow rate. The separation is accomplished by introducing a sample in liquid form onto a column, and then eluting the sample down the column with a liquid mobile phase continuously flushing through the column under pressure. The sample components are separated by their interactions with the mobile phase and column stationary phase. As the components are eluted off the column they pass through a detector and in some cases may then be collected.

12.2 ADVANTAGES AND LIMITATIONS

HPLC is a separation method that is useful in instances where the analytes are thermally unstable, or are not readily volatilized. It can be applied to many different analytes by selecting different columns and mobile phases, and can also be useful for collecting fractions of separated analytes, although this technique is not currently employed in the Crime Laboratory Division.

HPLC may be limited in its usefulness by short column lifetimes, the generation of large volumes of mobile phase waste (potentially hazardous), and the tendency for these systems to have mechanical problems. The short column lifetime and mechanical problems can be reduced through proper maintenance and sample preparation.

HPLC can be used for either qualitative or quantitative analysis. For quantitative analysis, there are several approaches that can be taken including: peak area or height normalization, external standard, internal standard and standard addition methods. The method selected will depend on the analytes of interest and detector used. The quantitative method used must be able to identify the component being quantitated and ensure the peak is pure (no coeluting peaks).

UV detection provides limited structural information so HPLC-DAD is regarded as a category 2 test for seized drug analysis. HPLC-DAD may be used for quantitative analysis with a validated method.

12.3 APPARATUS AND EQUIPMENT

HPLC systems consist of several components; mobile phase reservoir(s), pump, injector, column (with temperature controller), detector and waste collector. The mobile phase reservoirs hold a solvent or solvent mixture. If there is only one mobile phase reservoir the system mode must be isocratic (constant composition). With multiple reservoirs the system can be run in isocratic mode or using a gradient (changing composition). The currently used HPLC system is equipped with a UV diode array detection system (DAD). Other detection systems are commercially available.

HPLC grade solvents, or better, must be used for extractions, dilutions and mobile phases. Other commercially available HPLC reagents may also be used.

Mobile phases may be prepared in-house as needed or purchased from commercial vendors.

12.4 PROCEDURE

Sample preparation is typically done by diluting or homogenization via extraction, centrifugation and/or filtration of the sample with HPLC grade or better solvents.

Varying the size of the sample loop can be used to either increase or decrease the injection volume capacity. This can be used in conjunction with sample dilution to achieve an acceptable sample concentration for analysis.

Separation methods are selected that are appropriate for the analytes of interest. For example, seized drug or cannabinoid methods have been designed to separate specific species.
Analytical columns and mobile phases appropriate for the selected method are used. The column must not be allowed to dry out. A single analytical column may be used for more than one method.

The pump must be primed before use to remove air bubbles from the solvent lines. This should be done by opening the purge valve and running a high flow rate (2+ mL/min) for a few minutes prior to running samples.

Detection is accomplished with the UV DAD. Spectral information from 190 to 600 nm may be collected. The detector should be turned on at least 20 minutes before samples are run to ensure the detector is warm and help prevent baseline drift.

Each method is designed to monitor the separation at a wavelength appropriate for the analytes of interest.

Vial positions must be verified and documented.

Results will be recorded in the case notes and data included in the case file. The case file will also contain the following information for each sample run:

- Unique Identifier of the instrument used (unless the laboratory only has one instrument and that instruments identification is documented in the laboratory’s equipment list).
- The name of the method used
- The column temperature program
- Sample name
- Date and time
- The column stationary phase and physical dimensions
- Mobile phase profile
- Injection volume
- Detector monitoring wavelength

Other instrument conditions may be recorded in a logbook or other storage location as long as they are traceable and routine to the laboratory. If non-routine, these will be recorded in the case file.

12.5 INTERPRETATION

Analytical reference materials appropriate to the method being used and/or the analytes being examined must be run under the same analytical conditions in order to evaluate the sample data.

Peaks for seized drugs and other types of evidence will be evaluated for their significance and documented. The retention time of the sample should agree within $\pm$ 5% of the retention time of the reference material to be considered acceptable. When evaluating the retention times, consider injection methods, concentration, peak shape and sample preparation conditions. For example, a large difference in the concentration of the reference material and the sample will commonly lead to differences in the retention time. This can be remedied by diluting or concentrating the reference material or the sample and re-injecting to achieve retention time agreement between the reference material and the sample. Sample preparation methods and substances that elute over a wide range may lead to a variance of greater than the values given above. Any variance greater than the range specified must be further evaluated: it will be considered acceptable with adequate scientific justification, concurrence of the reviewer, and with the justification in the case notes.

Spectral data may assist in distinguishing compounds in simple mixtures or acid/neutral forms of cannabinoids especially during method development. When spectral data is used for analyte confirmation, the unknown analyte must be compared to the reference material both for relative migration time and spectral concurrence.

12.6 **QUALITY ASSURANCE**

12.6.1 CALIBRATION

HPLC systems do not require calibration.

12.6.2 MAINTENANCE

The HPLC system is maintained by following the user manual recommendations.

12.6.3 PERFORMANCE VERIFICATION

Individual analysis methods also need to be performance checked using reference materials. This must be done at least once each day when the method is in use and a record of the performance check must be included in the case file. An appropriate preparation blank must be run to performance check the extraction/dilution solvent(s) to demonstrate that they are free from interfering substances. HPLC (or comparable) grade solvents that pass the performance check may be used even if they have exceeded the manufacturer’s expiration date.

If the instrument is used only infrequently, it does not warrant as frequent a check of the lamp as the manufacturer specifies. Gaps between checks are not a concern during periods of inactivity (e.g., holidays), as long as the performance is checked prior to subsequent use in casework.

New instruments or instruments transferred from one lab to another will be verified by running a series of reference materials over at least a one week time period. The retention times of the reference materials will be evaluated for drift over the evaluation period. Data from the evaluation period will be kept with the verification paper work.

12.7 **SAFETY**

The scientist should be aware of any hazards associated with the chemicals used as mobile phases. Pressurized liquids can become airborne through leaks, and appropriate eye protection should be used.

Exposure to high powered UV can cause blindness and thermal burns.

13 IMAGING & VISUALIZATION

13.1 INTRODUCTION

Forensic examination sometimes requires specialized techniques to evaluate evidence for details that are difficult to see with the unaided eye. Two techniques with broad application in evidence examination are Alternate Light Source (ALS) or Forensic Light Source (FLS) and digital imaging technologies. An ALS/FLS is a specialized light that combines powerful illumination with adjustable, high-end visible wavelengths. Physical evidence, such as fibers and biological fluids, may fluoresce when exposed to this type of light. Digital imaging technology is used to preserve, and document various types of physical evidence, as well as an aid to further investigate evidence for non-visible information/details.

13.2 ADVANTAGES AND LIMITATIONS

ALS/FLS and digital imaging technology are non-destructive to the evidence (leaving it available for subsequent examinations) and they reveal characteristics of the evidence that may not be readily observable. The proper utilization of the equipment and techniques gives a scientist the ability to observe and document difficult-to-see stains and physical evidence.

ALS/FLS must be viewed in a darkened area which can limit its applicability in certain circumstances. Digital enhancement may be limited based on the quality of the original image.

13.3 APPARATUS AND EQUIPMENT

Visualization instruments and equipment varies from lab to lab but generally includes lighting sources, filters, hand-held ultraviolet (UV), infrared (IR) and ALS/FLS. Digital imaging technology also varies and may include digital SLR cameras, compact digital cameras, digital microscope cameras, IR/UV digital cameras, and digital imaging systems. Adobe® Photoshop® or other appropriate software is frequently used to duplicate images; adjust size, brightness, contrast, color, hue and saturation; and affix scales or magnification descriptors to images.

13.4 PROCEDURE

13.4.1 <u>ALS/FLS</u>

Examinations using ALS/FLS shall be conducted in a darkened room. Turn on the ALS/FLS and allow the lamp to warm up and select the appropriate wavelength. Adjust the power control knob (if equipped) to either increase or decrease the intensity of the light output. Appropriate colored goggles shall be worn (refer to the manufacturer's guidelines). Spread out the item to be examined on clean paper. Holding the light source approximately six to twelve inches from the item being examined, systematically scan the item for areas of fluorescence. Mark and/or note any areas of fluorescence for further testing or collect the fluorescing materials. Turn off the lamp when finished and allow the lamp to cool before turning off the unit (if this is a unit feature).

13.4.2 <u>IMAGE CAPTURE</u>

Image capture devices include compact digital cameras, digital SLRs, scanners, and other image capture systems (such as LEEDS and foster+freeman). The intended purpose of an image will indicate which type of device is most appropriate for use.

For documentation of general observations, a compact digital camera may be appropriate. Resolution and file type may vary for these images.

For images intended for examination or comparison of detail, an appropriate digital SLR camera, scanner or optical system with higher resolution should be used. The captured images should be recorded in lossless file formats (such as RAW or TIF) and compression should be avoided.

13.4.3 IMAGE ENHANCEMENT

Digital image enhancement is used to improve the appearance of an image, typically to optimize the image for best contrast. Digital enhancement may be utilized for both documentation and comparison type images.

Simple enhancements, such as contrast and brightness adjustments, for an image in which the image is not used in a comparison examination do not require saving an original and final image with documentation.

For enhancements of comparison-type images, a working copy should be created and saved with a unique file name. The working copy is processed and the original image remains unaltered. The enhancement technique(s) must be explainable, within the scope of the scientist's training, and reproducible. The enhancement techniques must be recorded in the case file, whether ~~it's~~ handwritten in the notes, included as an Adobe® Photoshop® log file, or imbedded in the image.

13.4.4 DIGITAL IMAGE STORAGE

Original digital image files will be saved to a permanent storage device. Approved digital media are to be used for storage. Images retained are considered part of the case record. Refer to CLD QOM 8.

When saving examination quality images to digital media, the same format in which they were captured should be used. The unaltered saved images will be considered the original images. If possible, the media will be marked with the date it was created, the CLD case number, scientist's initials.

All printed copies of digital images are considered working copies.

13.5 INTERPRETATION

Interpretation is addressed in the individual sub-discipline procedures.

13.6 QUALITY ASSURANCE

If an image is resized to approximate the natural size of the depicted subject, an appropriate measuring tool must be used to check the resizing and this check should be noted in the case file.

13.7 SAFETY

ALS/FLS is relatively safe but precautions should be taken to avoid looking directly at the sources as this can cause fluorescence of the eyeball which would result in decreased ability to detect the fluorescence response in the sample. Skin exposure to the ALS/FLS, especially when taking medications that produce increased photosensitivity, can lead to skin irritation. It is recommended that scientists wear long sleeves, non-fluorescent clothing and non-photochromic goggles with UV-blocking capabilities while using the ALS/FLS.

14 INFRARED SPECTROSCOPY

14.1 INTRODUCTION

Infrared spectroscopy is an analytical technique that provides structural information about a sample. It is based on the selective absorption of radiation in the mid-infrared region, which extends from 4000 wavenumbers (or reciprocal centimeters abbreviated as cm^{-1}) to ~250 wavenumbers.

Infrared spectroscopy can serve both to characterize an unknown substance, by providing information about the presence of functional groups in organic compounds or the presence of certain anions or cations in inorganic compounds, and to identify many compounds, by providing enough distinct spectral characteristics so that no other compound exhibits this particular pattern of absorptions. The latter is true of low to moderate molecular weight organic compounds (except for higher members of various homologous series) and low molecular weight inorganic compounds analyzed in the vapor phase under high resolutions. It also holds for some inorganic compounds, especially minerals and some other compounds comprised primarily of covalent type bonds, but this is not necessarily the case for some salts with a monoatomic cation or anion.

14.2 ADVANTAGES AND LIMITATIONS

Infrared spectroscopy has a very wide variety of applications since the only compounds that do not absorb in the mid-infrared region are homonuclear diatomic molecules and some simple inorganic salts. Solids, liquids, and vapors may all be readily analyzed. The method is an excellent means of determining the presence of functional groups in organic compounds, of obtaining information about the presence of many common inorganic cations or anions, and of determining the generic type of a polymeric substance. Fourier-Transform Infrared Spectroscopy (FT-IR) is regarded as a Category 1 test for seized drug analysis.

In comparison to gas chromatography/mass spectrometry (GC/MS), the other very widely-used method to identify organic compounds, infrared spectroscopy can be used to analyze non-volatile and heat-labile compounds, common diluents in drug exhibits that cannot be chromatographed using GC/MS, and common substitutes for drugs that cannot be chromatographed. Infrared spectroscopy also allows one to distinguish between different isomers, excluding enantiomers, as well as to determine the particular form that certain compounds are in.

IR analysis provides non-destructive, structural information for the qualitative identification of a wide range of substances, both organic and inorganic. Analytical strengths of IR include needing limited sample quantities for analysis and the ability to determine differences between diastereomers. Limitations include the need for samples to be relatively pure and the difficulty of identification within a homologous series or polymer group. The lack of spectral complexity may prevent specific identification of inorganic compounds as well.

14.3 APPARATUS AND EQUIPMENT

FT-IR spectrometers are single beam instruments and both a reference spectrum and a sample spectrum need to be acquired, then ratioed to determine the percent of each wavelength of infrared light that is absorbed (or transmitted). The raw data that is generated by an FT-IR instrument is an interferogram, which contains information about all of the infrared wavelengths reaching the detector. A mathematical process, a Fourier transform, is used to obtain this information from the interferogram. The specifics of how an FT-IR instrument operates is provided in the training given to scientists, as outlined in the Materials Analysis Primary Foundation Training Manual.

14.4 PROCEDURE

A number of different FT-IR sampling accessories and analysis techniques are currently used by the various WSP Crime Laboratories. They include: (1) Various microscopes and accompanying accessories (2) Diamond anvil cells used with beam condensers; (3) Single-pass micro ATR accessories, either with zinc selenide or diamond coated elements; (4) Vapor cells, including a variable multi-pass vapor cell; and (5) Various types of diffuse reflectance (DRIFTS) accessories. Because each of these has its own sample preparation requirements, advantages, limitations, features, and peculiarities, it is not practical to attempt to give detailed procedures for each case, particularly as an extremely wide variety of samples may be subject to analysis. Instead, the specifics of these will be covered when scientists are trained in the use of a particular accessory and in the general training module on infrared spectroscopy.

One important limitation of some accessories should be noted, however. For those accessories where only a mercury cadmium telluride (MCT) detector can be used (including the infrared microscope), there will necessarily be a more limited spectral range that can be examined since the low frequency cut-off points for MCT detectors occur between 750 and 450 cm^{-1} (and the value for almost all infrared microscopes and GC/FT-IR accessories is closer to 750 cm^{-1} since these almost always use narrow-band MCT detectors). Many inorganic oxides do not have any significant absorptions above 700 cm^{-1}, so these absorptions cannot be observed if an accessory using a narrow-band MCT detector is used. For many other inorganic compounds which do have absorptions above 700 cm^{-1}, there are often lower frequency absorptions below this value which are also very important in characterization and differentiation (for example various silicate minerals). The method of choice when analyzing most inorganic compounds, or materials containing inorganic compounds, is an extended range CsI FT-IR instrument, which can collect spectral data down to 250 cm^{-1}.

Regardless of what sample or accessory is used, scientists should collect enough scans so that an adequate signal to noise ratio is achieved and all of the important features of interest in the spectrum are clearly distinguishable from the noise levels

The scientist must verify the accessory used is free from contamination prior to obtaining a reference spectrum and sample spectrum.

Results will be recorded in the case notes and data included in the case file. When applicable, the compound’s salt form will be indicated in the case notes.

All case files should include sample spectra for both questioned and known samples. If sub-sample (e.g., individual particle) spectra are acquired they should be present for both the known and questioned samples. The spectra should be collected under similar conditions using the same protocol. If the analysis involves the comparison of a spectrum against a library standard, both spectra should be included. In all cases the major peaks must be identified on the spectra. For clarity, if the spectra are overlaid on the same page then only one set of peak labels should be annotated on the page. Most modern spectrometers allow for the production of spectra/reports with various sampling information annotated on the printed page. It is recommended that at a minimum the following information be included on any printed spectra:

- The number of sample scans
- The date and time of the sample scans
- Unique Identifier of the instrument used (unless the laboratory only has one instrument and that instrument’s identification is documented in the laboratory’s equipment list.)
- The accessory used (DAC/diamond anvil cell, etc.)
- The spectral resolution used
- The number of background scans recorded, if the instrument is capable of documenting this information

- The date and time of the background scans, if the instrument is capable of documenting this information

Any processing of the data, such as background corrections, subtractions, etc. will also be recorded on the printout.

14.5 INTERPRETATION

The physical state of a particular compound, that is, whether it is analyzed as a vapor, liquid, solution, or solid, may have a very strong bearing on the appearance of its infrared spectrum.

The interpretation of infrared spectra involves the correlation of absorption bands in the spectrum of an unknown compound with the absorption frequencies for known materials. Significant for the identification of the source of an absorption band are intensity (weak, medium or strong), shape (broad or sharp), and position (cm^{-1}) in the spectrum. In general, a spectrum for a questioned material and a known material or a reference standard should show correspondence in all areas mentioned above. However, there are many variables which may slightly alter the IR spectra. Many samples, such as paints, have pigments and other fillers which are not present if one compares the spectrum to a library spectrum. If the analysis involves the comparison of questioned and known samples, both of which were analyzed using the same protocol, it can be expected that there would be only very minor differences in the two spectra. For specific information on IR interpretation refer to the training manuals and technical procedures for the individual sub-disciplines.

When using infrared spectroscopy for identification, a peak to peak comparison of a pure reference material and sample spectra should be made, and any significant differences must be noted on the spectrum. Samples with spectra that have many extra absorptions likely contain another component(s), and scientists should utilize a scheme to isolate the compound of interest if further infrared data are sought. What constitutes acceptable spectra for identification, however, will depend on several factors, including the degree to which the spectra of the pure reference material and sample differ, the nature of the sample, and any supplemental information that the scientist may have collected regarding the identity of the component(s) responsible for the extra absorptions. The presence of weak extra peaks consistent with a compound that is also indicated by GC/MS data, for example, would be less of a concern than those of a component(s) that is unidentified. Since a continuum of values can occur for the number, frequencies, and intensities of extra absorptions in a spectrum, there are no simple criteria for indicating when a particular spectrum does not provide sufficient individualizing characteristics for identification. Without further supporting data, scientists should thus take a conservative approach when attaching significance to such spectra and proceed with their analysis accordingly.

When possible, comparisons between sample and reference spectra should be made for data collected on the same instrument with the same accessory. A copy of the reference will be included in the case file.

14.6 QUALITY ASSURANCE

The instrument will have a logbook to document, at a minimum, maintenance (excluding routine cleaning), results of performance verifications, damage, malfunctions, modifications, repairs, and when the instrument is placed out of service and returned to service. Each of these points will include the relevant date(s) and person(s).

14.6.1 CALIBRATION

The FT-IR does not require user calibration methods. The helium-neon laser of an FT-IR spectrometer serves as a calibration device that is in continuous operation during every scan. FT-IR instruments are thus self-calibrating and if the laser fails or its output diminishes to the point where the laser interferogram can no longer be read, the instrument will not operate.

14.6.2 MAINTENANCE

The FT-IR is a robust instrument that requires minimal user maintenance. This maintenance may include routine cleaning, desiccant changes, and part replacement as appropriate. Routine cleaning will include keeping the sampling area clean.

Outside service or any maintenance (excluding routine cleaning) to any part or accessory of the instrument must be recorded in the logbook.

Because an FT-IR has so few moving parts, the only items that normally need to be replaced are the infrared source and the helium-neon laser. Instruments equipped with MCT detectors will also require a periodic re-evacuation (probably after five to seven years), as the MCT crystal is cooled with liquid nitrogen and must be kept in a vacuum to prevent condensation. When this re-evacuation procedure needs to be performed will be evident because the liquid nitrogen dewar will have to be refilled every two or three hours, instead of lasting eight hours or longer when the detector is properly evacuated.

To protect the optics of an FT-IR instrument, it is recommended that FT-IR spectrometers be purged with dry and carbon dioxide-free air. Instruments with sealed optics do not require purging.

14.6.3 PERFORMANCE VERIFICATION

The performance of the instrument will be verified using the manufacturer's software.

The Seattle Laboratory GC/FT-IR instrument does not have a detector on the bench portion and the absorption bands of cocaine run on the GC/FT-IR at 2968, 1736, 1274, 1113, and 710 cm^{-1} are monitored when the weekly drug mix is run. The observed frequencies should be within ±4 cm^{-1} of these values to pass. If any of these tests indicate that an instrument adjustment (particularly an alignment) is needed, it should be conducted and the diagnostic check rerun. Gaps between checks are not a concern during periods of inactivity (e.g., holidays) so long as the performance is checked prior to subsequent use. These diagnostics should also be run following any instrument maintenance or as needed based on the discretion of the user. GC retention times of the weekly drug mix should be within 2% of those of the previous run.

A series of reference materials will be evaluated on a new instrument or an instrument transferred from another lab. Spectral data of the reference materials will be compared to literature and documentation of these comparisons will be kept with the verification paper work.

14.7 **SAFETY**

Appropriate safety precautions should be employed when refilling the liquid nitrogen dewar on instruments equipped with an MCT detector. Personal protective equipment including safety goggles, face shields, insulating gloves and long sleeves should be used when handling liquid nitrogen.

15 MICROCHEMICAL TESTING

15.1 INTRODUCTION

Chemical color tests are generally nonspecific screening tests that provide preliminary data as to the nature of the substance to be identified. Certain compounds or classes of compounds produce distinct colors or precipitates when brought into contact with various chemical reagents. These simple reactions can indicate the presence of a particular functional group or a molecular moiety, but are not used alone to conclusively identify the presence of any specific drug or chemical. Color tests are empirical in nature; basic theory is available in the references listed in the training manual.

Microcrystalline tests are chemical-precipitation tests in which a light microscope is used to identify individual chemical substances by their specific crystalline formations. They are based on the formation of an insoluble salt or complex of the chemical substance of interest with a reagent, thus forming characteristic crystals. Microcrystal tests are very specific for some chemical substances, can distinguish isomers in some cases, and can yield crystals with many chemical substances that are present in low concentrations.

15.2 ADVANTAGES AND LIMITATIONS

Microchemical tests are fast, require very little sample, may be non-destructive (in that the tested material might be recoverable), can be used to separate components from some mixtures or distinguish among the isomers of some compounds and are highly specific for many compounds. Some disadvantages are that when first starting the scientist must test many compounds to learn what the products are and which reagents work best. The scientist must learn how to be consistent in reporting what is seen, i.e. the written description or drawings of the crystals must be reported in the same manner from case to case. There is no print-out of the results (although digital photomicrography may be used for documentation) and some closely related compounds may give the same type of crystals.

Color tests are often instantaneous. Most of the reagents are inexpensive, store indefinitely and are easy to handle with a simple apparatus. There is abundant documentation about these tests. Moreover, there is a rich legacy of mechanistic organic chemistry which backs up the conclusions provided by these tests, and instrumental analysis is further strengthening the understanding of them.

It is often impossible to identify a substance to the exclusion of all others (particularly homologs or stereoisomers) using color tests. Pure samples often require very little material (1 mg or less), but complex matrices in which the active ingredient is a minor component require larger portions for distinct results. Mixtures can lead to data that is difficult to interpret. This can be overcome in conjunction with thin-layer chromatography, which often uses these color reagents as stains.

There is always a certain amount of subjectivity that must be taken into account when a color is reported. It is not uncommon for two scientists to describe the same color differently. Aside from the differences in reporting colors that can be attributed to the scientist, colors can also be influenced by the concentration of the sample in the reagent, by the presence of contaminants, or by the age of the reagent. Also, the length of time during which the colors are observed may influence the color reported because color transitions and instabilities are not unusual. Allowances should, therefore, be made for these differences, especially with evidence items where neither the concentration of the chemical nor the presence or composition of any contaminant is known.

Microcrystal tests are rapid and generally very sensitive. The tests are highly specific for certain drugs as a limited number of chemical substances will produce unique crystal habits.

Microcrystal tests work well for many chemical substances including compounds containing basic nitrogen groups. There is frequently no isolation/extraction step required as most diluents do not interfere. In some cases, the tests can be used to distinguish pure enantiomeric isomers from racemic mixtures.

Some mixtures will alter the crystal habit or prevent the crystal formation for certain chemical substances. For example, excipient material may distort crystal formation or structure, or one may see mixed crystals. Furthermore, closely related compounds often do not form any crystals. Some crystal tests are not very specific for certain classes of drugs. In general, this technique is a destructive analytical method.

15.3 APPARATUS AND EQUIPMENT

In general the following items should be available:

- Cover slips
- Filter paper
- Glass stirring rods
- Glass wool
- Laboratory wipes
- Microscope slides
- Microscopes
- Pipettes (glass, plastic, various sizes)
- Reagent bottles
- Reagents (see training manual for specifics and formulas)
- Reference materials
- Spatulas
- Spot Plates (white and black or another dark color)
- Tungsten needles

15.4 PROCEDURE

Prior to conducting any microchemical test the scientist should be familiar with the procedures of that test, its limitations, interfering materials, sensitivity, etc. Tests must be conducted as described in the training manuals or in published reference materials.

For all types of microchemical tests the scientist should document what they see. Descriptions, sketches or photographs of the crystals will be used to document the results of the test. A blank must be performed and documented with each test.

Color tests are generally conducted by adding one to two drops of reagent to a well on a spot plate. This serves as the blank prior to adding a small amount of the substance to be tested. Any color which is observed after adding the sample and the results of the blank are recorded in the case notes. Several chemical screening tests involve a modification of this general procedure. The Seized Drugs and Secondary Foundation Training Manuals or other publications describe modifications to this general procedure.

Numerous microcrystalline test methods are documented in the scientific literature and are available to be used for microchemical testing. For testing, a small quantity of the microcrystalline reagent is added to the sample or a solution of the sample dissolved in an appropriate medium (acid or solvent). Any resulting crystals are observed using a polarized light microscope. As you examine the crystals and rotate the stage, the relief, the way they “appear” and “disappear”, and the way the crystals “change colors” give you additional information. Even no reaction, or the formation of a precipitate but no crystals, tells you something about the material being tested. When definite crystals have been formed, a written description or sketch must be noted.

Refer to the Biochemical Analysis Procedures for information regarding presumptive blood testing.

15.5 **INTERPRETATION**

The scientist must be familiar with the microchemical test(s) they are using in order to appropriately interpret the results. Interpretation is based on expected results versus the actual result. Interfering materials or compounds of a similar class must be considered when interpreting results.

The interpretation of a color test~~s~~ depends on a change in color or lack of color change, it may also involve effervescence and/or precipitation. These observations can be compared directly to the reaction of a reference material, results from past experience, or descriptions of results from publications.

Experience is extremely critical in the interpretation of crystal formations. A quick glance is not sufficient, as one must know how to observe crystals carefully and with attention to detail. Interpretation of microcrystalline tests depends on the comparison of the crystals formed from the unknown with those prepared from a reference material and the same reagent. Impurities in the test solution may lead to the formation of deformed, irregular or unusual crystals, but this can be overcome by utilizing clean up procedures such as extractions or particle picking. Polymorphism may also interfere with crystal interpretations. The most common differences arise from the fact that the appearance of the crystals may depend on the concentration of the solution from which they are formed.

15.6 **QUALITY ASSURANCE**

Chemicals and reagents used in microchemical testing must be of the appropriate grade for the test performed. For most test procedures, reagent grade chemicals are sufficient unless specifically stated otherwise in the individual sub-discipline technical procedures. Written formulations for all chemical reagents used in the laboratory must be available and should include the reference for the test procedure, a description of what the test is used for, and the preparation of the reagent(s).

Many microchemical testing solutions can be made up and stored indefinitely. Scientists should be aware of the shelf life of reagents. Reagents shall be labeled with, at a minimum, the identity of the reagent and the date of preparation or lot number. Newly prepared reagents will be verified by running a negative control and a positive control using a reference material. These results will be recorded in a reagent logbook. Log books shall be maintained identifying the preparer of the reagent and the results of the reliability check of the reagent. The log book entry describing reference materials used to test the reagents must include their source and lot number (if available) or laboratory identification number. One time use reagents need only be documented in the case notes.

For reagents used in seized drug testing, monthly checks must be performed using reference materials. Chemical screening tests which are not considered a Seized Drug Analysis Category 1 or Category 2 test will be reliability checked when prepared and as appropriate. Gaps between checks are not a concern during periods of inactivity so long as the performance is checked prior to subsequent use. These checks will be documented in the reagent log book.

For other types of microchemical reagents the specific reliability checks will be addressed in the sub-discipline technical procedures.

15.7 SAFETY

It is important to note that some of the ingredients in microchemical testing reagents pose significant health hazards and before making or using any of the reagents, the appropriate SDS should be consulted. Appropriate precautions, including use of personal protective equipment and fume hoods, should be utilized.

16 MICROSCOPES

16.1 INTRODUCTION

Microscopes are used to locate, characterize, identify, compare and/or test various types of evidence. Examinations are based on microscopic morphological features, chemical and physical characteristics and properties, and the components that make up these materials.

The following knowledge and skills are necessary for microscopic examination of evidence:

- Small particle manipulation.
- Experience in the use of stereomicroscopy and polarized light microscopy as an analytical tool.
- Experience in microchemical and solubility methods and techniques.
- Knowledge of the various instruments available to the examiner as an adjunct to microscopic methods.

16.2 ADVANTAGES AND LIMITATIONS

Many techniques and tests are specific for the materials being tested. There are several general procedures available for the analysis and characterization of evidence exhibits, some of which are non-destructive. A properly prepared scheme of analysis will allow several tests to be conducted before the sample is consumed in certain cases. The investigator's request, and the nature of the material involved will dictate the approaches, methods, and testing to be utilized.

Other than the initial cost of the microscope and accessories, the cost of consumables and maintenance is relatively low.

With the use of digital photography it is relatively easy to document the results of the testing and/or observations.

Extensive training is required to become and maintain proficiency as a microscopist. As a new microscopist it may take longer to conduct an examination because of the inexperience factor.

Without digital photography the scientist must be able to completely describe the results of testing methods in order for the reviewer to understand what was observed and the results of those observations.

16.3 APPARATUS AND EQUIPMENT

16.3.1 <u>STEREOMICROSCOPES</u>

Stereomicroscopes are binocular microscopes in which the object is observed by each eye from a slightly different angle with and have a magnification range commonly from 4 to 100X. A wide number of models, types and configurations are present in our system and are used for this function.

Considering the wide range of accessories currently available for stereomicroscope systems, this class of microscopes is extremely useful in a multitude of applications. Stands and illuminating bases for a variety of contrast enhancement techniques are available and can be adapted to virtually any working situation. There are a wide choice of objectives and eyepieces, enhanced with attachment lenses and coaxial illuminators that are fitted to the microscope as an intermediate tube. Working distances can range from three-five centimeters to as much as twenty centimeters in some models, allowing for a considerable amount of working room between the objective and specimen.

16.3.2 COMPOUND MICROSCOPES

The various configurations available for use in compound microscopes include polarized light, phase-contrast, reflected light, dark field, dispersion staining, and fluorescence. Various accessories may be used to tailor the microscope to the type(s) of examination(s) needed. Such accessories include wave plates (compensators), filters, eyepiece reticles, rotating stages, temperature controlled stages (hot-stage), condensers, objectives, light sources, etc.

16.3.3 COMPARISON MICROSCOPES

Comparison microscopes are a configuration of two microscopes connected by an optical bridge with a single head that allows the scientist to observe two samples simultaneously. The microscopes and accessories described in the previous sections apply to the configurations of the comparison microscope. For two samples to be labeled as “similar” with a comparison microscope, the samples must be prepared in the same way and observed under the same microscope conditions (objective lens, condenser aperture setting, filters, lighting intensity, etc.).

16.3.4 SPECIAL USE ACCESSORIES

Special use accessories include a Berek compensator, a Sénarmount compensator, a thermal stage (hot stage), a quartz wedge, and a dispersion lens objective.

16.3.5 MOUNTING MEDIA

Mounting media are used to enhance contrast of the specimen and the medium, used to determine refractive indices of material, and to provide a permanent or semi-permanent mount of the specimens. The sample determines which one(s) will be used. Care should be taken to ensure that in certain instances the specimen is not soluble in the media selected. As long as thismedium maintains its fluidity (with the aid of additional solvents) and does not affect the quality of the test, it can be used in examination after it has exceeded its expiration date. (Note, this is not intended to be a comprehensive list of all mounting media that could be used.)

16.3.6 PERMOUNT® MOUNTING MEDIA (FISHER SCIENTIFIC)

A good general mounting medium. Its refractive index at 20ºC ranges from 1.518 to 1.521 when the solvent has evaporated. If solvent is still present, the refractive index may be lower. It is good for making semi-permanent mounts (the sample can be removed from the medium at a later time with a solvent such as xylene or toluene). It should be noted that a possibility exists for the Permount® to alter the color and/or slightly dissolve the mounted material.

16.3.7 NORLAND OPTICAL ADHESIVE

An excellent permanent mounting medium. The company has the mounting medium available in three refractive indices: Norland 61, refractive index-1.560; Norland 65, refractive index-1.524; Norland 68, refractive index-1.540. The medium stays viscous until it is cured with an approximately 3 minute exposure to UV light. Norland mounting media would be best used for making permanent mounts of standards and reference material. Once cured it is almost impossible to recover the sample.

16.3.8 CARGILLE REFRACTIVE INDEX LIQUIDS

An excellent non-permanent series of mounting media. They are certified and can be acquired in ranges from 1.300 to 2.31 in various increments. They are critical for refractive index determinations (with and without a hot stage) and dispersion staining.

16.3.9 CARGILLE MELTMOUNT® MOUNTING MEDIA

A series of mounting media consisting of optical quality thermoplastics for making permanent microscope slides. It is available in either 1 oz. jars that can be placed directly on a hotplate, or in 2/3 oz. Quickstick® form that can be applied directly to a slide on a hotplate. It is available in refractive indices ranging from 1.539 to 1.704.

16.3.10 GLYCERIN JELLY
A mounting medium which can be used to make permanent mounts of plant material. It can be applied to pre-stained specimens or a stain can be mixed with the glycerin jelly and applied to the specimen.

16.3.11 CLEAR FINGERNAIL POLISH
Used for making scale casts of animal hairs.

16.3.12 NEO-CLEAR®
Neo-Clear® is an aliphatic hydrocarbon mixture that can be used in place of xylene as a temporary mounting medium.

16.4 PROCEDURE

The microscopes, accessories, and techniques used are as varied as the materials being tested. The scientist should be familiar with the basic microscopy and associated techniques for the sub-disciplines in which they are working.

Lenses are cleaned as needed prior to and/or during use. These include condenser lenses, objective lenses and eyepieces.

Ensure that the microscope is correctly aligned, has high intensity homogenous illumination and is focused correctly.

Depending on a particular microscope and its design, setting up Köhler illumination may be possible in full or only in part. Many modern microscopes are fitted with fixed light sources, which mean that it is not possible to center or focus the image of the lamp filament like in conventional microscopes. Also, many modern microscope designs include one or more diffuser filters between the lamp and the condenser. In such cases, even if the microscope is fitted with an adjustable lamp, an image of the filament cannot be seen. In these microscopes only partial Köhler illumination can be obtained. For microscopes capable of Köhler illumination, this alignment is a multi-step process which should be performed or checked as necessary. Such illumination can be set up following procedures described in the Materials Analysis Primary Foundation Training Manual and in various references.

Cameras may be attached to any of the microscopes. Such cameras may attach by an adapter that fits on an eyepiece, by an adapter that fits into the eyepiece tube when the eyepiece has been removed, or through an adapter that fits onto the third observation tube for those microscopes having a trinocular head. Specific requirements for digital imaging can be found in the QOM, Section 8.Digital Images in Casework Documentation.

16.5 QUALITY ASSURANCE

16.5.1 LOGBOOKS
Microscopes and accessories may be recorded in a single logbook or divided up into different logbooks depending on the needs of the particular laboratory. Any maintenance (excluding routine cleaning) to the microscope or to an accessory specific to that microscope must be recorded in the logbook. Outside service, change of lamp filaments, or calibration of eyepiece reticles for a specific microscope also must be recorded in the logbook.

16.5.2 EYEPIECE RETICLE CALIBRATION
When taking measurements on the microscope the eyepiece reticle must be calibrated with a stage micrometer. Digital imaging software used to make linear measurements on the photomicrographs will also be calibrated according to the manufacturer instructions. The stage micrometer will be calibrated to a traceable standard prior to use if not accompanied by a calibration certificate upon purchase. Any damage or other incident that may affect the calibration of the stage micrometer will require replacement or recalibration.

The eyepiece reticles must be recalibrated when the objectives are removed or replaced; when any component of the optical train is removed or replaced; or when the microscope is subjected to shock, jarring or bumping during use or after being moved.

NOTE: Every microscope used does not need to have the eyepiece reticle calibrated, just the ones used to take measurements. Microscopes in the section should be marked as to which ones are calibrated.

16.5.3 MAINTENANCE

Microscopes and accessories require very little outside maintenance. Most of the user maintenance on the microscopes and accessories will consist of preventive measures and routine cleaning. Occasionally, routine maintenance such as tightening loose screws can be performed by section personnel. When a problem is encountered with a microscope, the scientist should consult with a more experienced microscopist to see if the issue can be resolved. If the problem cannot be resolved, the supervisor should be notified and an outside vendor be contacted for repairs.

With comparison microscopes, replace lamps on both microscopes when one burns out. Both microscopes lamps should be on during use so filaments wear uniformly. Refer to the microscope owner's manual for additional information on the specific microscope used.

Preventive measures include:

- Turning off the light source and cover the microscope when not in use.
- Avoiding direct contact with corrosive chemicals.

Routine cleaning includes:

- Wiping the immersion oil off the 100x objective and sub-stage condenser immediately after use with a dry cotton swab followed by an alcohol swab. A clean piece of lens paper may be used instead of a cotton swab.
- Cleaning the exterior lens surfaces when needed using lens cleaner and/or a solvent such as alcohol.
- Do not use acetone, xylene, or other similar solvents to clean lens surfaces as these solvents may dissolve or loosen adhesives used to hold lenses in place.

16.5.4 PERFORMANCE VERIFICATIONS

Performance verifications are not required for microscopes and accessories because the image itself indicates whether the equipment is working properly.

16.6 **SAFETY**

Many of the microscopes are heavy; take proper precautions when moving them.

When conducting fluorescence microscopy, wear the proper eye protection or use appropriate filters when working around the microscope or viewing the sample through the eyepiece.

To remove or adjust lamps, disconnect the power and wear protective eyewear and gloves to prevent cuts and/or burns.

17 MICROSPECTROPHOTOMETRY

17.1 INTRODUCTION

A microspectrophotometer (MSP) is an instrument that combines the magnification capabilities of a microscope with the quantitative light measurement capabilities of a spectrophotometer. It is used to compare reflection or transmission properties of two materials as a function of wavelength (the spectral pattern). The spectrophotometer can measure the ultraviolet, visible, and near infrared regions (UV-VIS-NIR range) of the electromagnetic spectrum. The microscope is equipped with transmitted white light, reflected white light, and epifluorescence optics.

17.2 ADVANTAGES AND LIMITATIONS

An advantage of MSP is that it is a non-destructive, objective comparison of color. Because MSP is a microscopic method, the color components of very small fragments can be compared, or prescreened and then compared. It can distinguish metameric samples which may be visually indistinguishable. It is especially useful for dyed materials because intense color can be produced with only small amounts of dye. The low levels of dye in forensic samples are often not detected by infrared spectroscopy, whereas MSP may detect the color produced. In addition to dyed materials, the MSP may detect colored products from pigmented materials and from materials treated with fluorescent brighteners.

A limitation of MSP is that, in general, the UV-VIS-NIR range provides only limited structural information which is insufficient to identify chemical components. MSP may not be suitable for some very pale samples. MSP may have limited value for samples with limited spectral details.

17.3 APPARATUS AND EQUIPMENT

The microscope portion of the MSP is equipped with multiple objective lenses and transmittance, reflectance, and epifluorescence optics. The microscope illuminates the sample, permitting the analyst to select the area to be examined. The spectrometer receives light from the microscope through an aperture and separates the light into its various wavelengths with a diffraction grating. The specific wavelengths are then detected by a thermoelectrically cooled, charged couple device (CCD) array detector.

17.4 PROCEDURE

The microscope will be aligned and optimized for maximum signal throughput of the spectrometer. Samples that are to be compared to each other must be examined using the same sampling method. This includes the same mounting support (down to the type and thickness of the coverslip), mounting medium, objective lens, light path settings (such as substage diaphragm settings, filters, and collection time), and optics (eg reflectance, transmittance or fluorescence). Commonly used mounting supports include glass slides with glass coverslips, quartz slides with quartz coverslips, an open face diamond anvil cell, or tape lifts on transparency films. Commonly used mounting media include Cargille refractive index liquids, glycerin, Permount®, xylenes and xylene substitutes, or clear tapes. It is preferable to use a medium whose refractive index approximates the nominal refractive index of the sample to minimize changes in the light path at the sample/medium interface.

Transmittance Optics

> For samples examined with transmitted light, the data will be displayed in absorbance. When using glass slides, the range of wavelengths should be 400 nm to 760 nm to avoid spectral anomalies that may occur beyond this range. The light source must be one with a relatively even intensity across the visible range (xenon or LED). A reflective-type objective lens is recommended to minimize chromatic aberrations. The electronic noise

background (dark scan) and sample background (reference scan) will be collected for the same amount of time as the sample spectra. At minimum, a new dark scan and a new background (reference scan) will be taken for each separate mounting support and medium. If anomalous data is observed, the sample should be reanalyzed with additional dark scans and reference scans interjected as there are factors related to either the sample or the instrument which can cause drifting issues. More frequent dark scans control for instrument anomalies. More frequent reference scans control for sample anomalies. In some instances, a new reference scan may be needed prior to each sample scan. The examiner must also avoid unnecessarily long exposure of photosensitive samples and samples mounted on glass slides to the xenon light source as photo-bleaching of the sample and/or a gradual change in slope may occur. The shutter in the light path should be closed when not actively examining a sample to minimize these effects.

Reflectance Optics

Reflected light should not be used if suitable data can be collected with transmitted light. If reflectance is the only way to obtain spectra, the samples to be compared must have the same surface topology. The light source must be one with a relatively even intensity across the visible range (xenon or LED). The samples should not be prepared in a mounting medium, and the mounting support should not interfere with the optical path. A reflective-type objective lens must be used. The electronic noise background (dark scan) and sample background (reference scan) will be collected for the same amount of time as the sample spectra. Care should be taken to select an appropriate material for the sample background. If samples are to be compared, the material for the background must be the same for both samples.

Epifluorescence Optics

The light source must be one of high intensity (mercury or LED). A refractive-type objective lens is recommended because of the greater light throughput with such lenses. The electronic noise background (dark scan) will be collected for the same amount of time as the sample spectra. A reference spectrum will not be collected. Exposure time and use of shutters should be carefully considered to minimize photo-bleaching of dyes.

When collecting fluorescence spectra, care should be used in selecting a mounting medium to minimize autofluorescence. Spectra may still be collected from samples mounted in an autofluorescence mounting medium. In such situations, the color and/or intensity of the autofluorescence of the mounting medium must be different than the sample.

In situations with a large number of similarly-colored materials, the MSP may be used as a screening tool to locate appropriate samples for comparison against a target spectrum. A printout of the target spectrum will be included in the case notes. The screening spectra do not need to be saved; however, screened spectra are limited as “quick checks” of a sample and therefore are not sufficient for a comparison.

For comparisons with transmittance and reflectance optics, multiple spectra from the same sample should be collected to account for variations throughout a single sample and to compare separate samples. The number of spectra collected should be determined by the requirements of the specific sub-discipline; however, it is recommended that ten spectra be collected from each sample if possible. Spectra are to be printed out and included in the case file. Sampling method, sample collection time, and results of comparisons will be recorded in the case notes and/or printouts. For transmittance optics, statistical information such as averages and standard deviations must be calculated from absorbance data. For reflectance optics, the material used for the sample background must be the same for all spectra to be compared, and the material must be recorded in the case notes.

For comparisons with epifluorescence optics, the sample should be observed with all available cubes. A single spectrum may be collected from each sample with each cube that exhibits fluorescence. If more than one fluorophore is suspected, then multiple spectra may need to be taken. If the mounting medium exhibits autofluorescence with the same cube as the sample, then a spectrum of the mounting medium will need to be collected in addition to the sample spectrum. Spectra are to be printed out and included in the case file. Sampling method, sample collection time, and results of comparisons will be recorded in the case notes.

17.5 INTERPRETATION

Interpretation of spectra is based on two pieces of information: the spectral pattern and the intensity range of that pattern. The spectral pattern is related to the types of compounds present in the sample and is comprised of the location of the peaks and valleys, the relative intensity of peaks and valleys to each other, the presence or absence of shouldering, and the changes in slope between peaks and valleys. The intensity is the amount of photometric counts for any specific wavelength. The type of mounting support must be taken into account in determining the wavelength range that will be used for interpretation. Samples collected in the reflectance mode must be evaluated very carefully as sample surface effects can alter the spectrum.

For screening, the analyst need only consider the spectral pattern.

For comparisons of transmittance or reflectance data, the analyst must evaluate the multiple spectra from a sample to determine the amount of heterogeneity of the spectral pattern and the range of intensity for each pattern. If two different spectral patterns are present in the same sample, each pattern should be treated separately and additional spectra should be collected if possible to compare 10 spectra for each pattern. Once the amount of heterogeneity is determined, one of two methods may be used to compare the samples. Samples that have a high amount of heterogeneity and a broad range of intensity may be better analyzed by range overlap. Samples with low amounts of heterogeneity and a narrow range of intensity may be better analyzed by averages.

For comparisons by range overlap, multiple spectra from each of the questioned and known samples must be on the same printout. Samples compared by range overlap are similar if (1) the spectral patterns of the questioned and known samples are the same, and (2) at least one of the questioned spectra falls in the same range of intensity as one of the known spectra. This method of comparison is typically used for hand sections and peels of paint as well as for natural fibers.

For comparisons by averages, the spectral average from the questioned and the known samples must be on the same printout. Samples compared by averages are similar if (1) the spectral patterns between the questioned and known sample are the same, and (2) the intensity of the questioned spectral average is within 1 standard deviation of the known spectral average. This method of comparison is typically used for man-made fibers.

For comparisons of epifluorescence data, the spectra from each of the questioned and known samples from the same cube must be on the same printout. The compared samples are similar if the spectral patterns of the questioned and known samples are the same.

Consideration should be given to potential environmental exposure differences when questioned and known samples are determined to be dissimilar. Prolonged exposure to UV light or degradation from microbial, chemical, moisture, or other sources may alter the chemistry of the sample, particularly any dyes or fluorescent compounds. Questioned and known samples with different environmental exposure histories might be dissimilar, even if the questioned sample originated from the known.

17.6 **QUALITY ASSURANCE**

The instrument will have a logbook to document, at a minimum, the days the instrument is used, maintenance (excluding routine cleaning), results of performance verifications, shipping and return of reference standards, entry of recertification values of reference standards, damage, malfunctions, modifications, repairs, and when the instrument is taken out of service or placed back in service. Each of these points will include the relevant date(s) and person(s).

17.6.1 CALIBRATION

The MSP instrument is calibrated by the user every 2 years. The instrument will be marked as out of service during this process. First, the reference standards used for performance verifications of transmitted optics will be recertified by the manufacturer using a dedicated microspectrometer and NIST Standard Reference Materials. The shipping date and return date of the reference standards will be included in the logbook. Following recertification, the reference standards and their associated calibration certificates will be assessed. Documentation of this assessment is done via the Calibration Check form which will be maintained in either electronic or paper format. Finally, the instrument performance verification software will be updated with the values on the new certificate. The date the new values were entered will be included in the logbook. The instrument will be placed back into service upon passing the performance verification with the new values.

17.6.2 MAINTENANCE

The MSP is a robust instrument that requires minimal user maintenance and no manufacturer's maintenance. User maintenance will be performed on as needed basis and is limited to routine cleaning and replacement of microscope lamps. Routine cleaning will include keeping the microscope and optics clean. Exterior lens surfaces may be cleaned using lens cleaner, methanol, and/or other alcohols.

Outside service, change of lamp filaments, or any maintenance (excluding routine cleaning) to any part or accessory of the instrument must be recorded in the logbook.

17.6.3 PERFORMANCE VERIFICATIONS

The instrument verification must be performed each day of casework use prior to the casework sample. The verification will consist of wavelength and photometric checks of the detector with transmitted optics, using reference standards recommended by the manufacturer. A fluorescence check also must be run each day that the instrument is used to observe fluorescence. The fluorescence check will consist of spectra using each fluorescence cube with a designated reference material(s). Printouts of the wavelength, photometric, and fluorescence checks will be kept in a record notebook.

17.7 **SAFETY**

Care should be taken to avoid looking directly at light coming from the lamps as eye damage can occur. The lamps also use high voltage power supplies that could result in serious electrical shock if contacted while on. They also generate significant heat so care should be taken not to contact them while in operation. Also, replacing the dust cover over the instrument should not be done until the lamps have cooled down. To remove lamps, disconnect the power and wear protective eyewear and gloves to prevent cuts and/or burns.

18 PIPETTES

18.1 INTRODUCTION

Accurately pipetting volumes of liquid is a critical step in quantitative analysis. While several different models of pipettes may be available throughout the chemistry functional area, this procedure is intended to apply to the positive displacement pipettes used for quantitative analysis.

18.2 APPARATUS AND EQUIPMENT

- Positive displacement pipettes
- Capillary and piston assembling of size corresponding to the pipette
- Bottle top dispensers
- Evaporation trap

18.3 ADVANTAGES AND LIMITATIONS

Positive displacement pipettes utilize a disposable plunger for displacing fluid. The effects of viscosity and the specific gravity of the solution are minimized compared to air displacement pipettes. Positive displacement pipettes are the most accurate type of pipettes.

The performance of the pipette is most greatly influenced by the training and experience of the pipette operator.

Bottle top dispensers accurately distribute larger volumes of solvent directly from the storage bottle minimizing solvent transfer and handling.

18.4 PROCEDURE

Each day of use the pipette should be inspected to ensure it is free from dust and dirt. Wipe the pipette with a 70% ethanol solution if needed.

Liquids and pipettes should be equilibrated to room temperature prior to pipetting.

Use the forward pipetting technique for volatile liquids.

To improve accuracy, hold the pipette vertical and immerse the pipette tip to the proper depth depending on the volume of the pipette being used.

Pre-rinse the pipette tip one to three times with the liquid to ensure accuracy. Carefully check for air bubbles in the tip prior to dispensing a sample.

Store pipettes in an upright position when not in use.

Follow the manufacturer recommendations for installation and purging air from the bottle top dispenser.

Raise the piston slowly and evenly to the top to pull solvent into the cylinder for bottle top dispensers. Push down slowly and evenly to dispel the liquid into the collection vessel.

18.5 QUALITY ASSURANCE

18.5.1 CALIBRATION

Pipettes and bottle top dispensers used in quantitation casework will be calibrated by an external calibration service at least once a calendar year. The calibration certificates issued will contain the measurement results “as found” and “as returned” and will include the measurement uncertainty and/or a statement of compliance with an identified metrological specification.

The hygrometer/thermometer/barometer will be calibrated by an ISO17025 accredited calibration laboratory and will be re-calibrated prior to the expiration dated listed on the calibration certificate. The calibration due date will be labeled on the instrument.

Following calibration the pipettes, bottle top dispensers, hygrometer/thermometer/barometer, and their associated calibration certificates will be assessed before the equipment is placed back in service. Documentation of this assessment is done via the Calibration Check form which will be maintained in either electronic or paper format.

18.5.2 MAINTENANCE

An authorized vendor will evaluate and perform necessary maintenance and repair on pipettes.

18.5.3 PERFORMANCE VERFICATION

Pipettes and bottle top dispensers used in quantitation casework will be monitored monthly using the Calibry software. Prior to pipette monitoring the balance and evaporation trap will be allowed to reach equilibrium (approximately two hours) after set up. Temperature, humidity, and barometric pressure will be determined using a NIST traceable thermometer/hygrometer/barometer and recorded in the Calibry software. Default ISO parameters will be selected for those pipettes pre-populated in the database. For pipettes or bottle top dispensers that must be added to the software, systematic and random error will be set at 2% for the specified volume. Four measurements at a minimum of two volumes will be selected for monitoring. The software method created for new pipettes will be verified by someone other than the scientist who created the method. Documentation of this verification will be made in the Calibry software in the following location: Select the “Options” menu. Select “Calibration Set Up”. Select “Notes”. Select “Add”. Type the description of the method, who verified the method and the date of the verification”. Select “Apply”.

Pipettes and dispensers that do not pass this check will be rechecked. If the pipette or dispenser does not pass the check by the second scientist, the device will be taken out of service and sent to the contracted pipette service provider for service and calibration.

18.6 **SAFETY**

Maintaining good posture and taking frequent breaks will reduce the likelihood of repetitive strain injuries which are frequently attributed to repetitive pipetting.

Do not carry a mounted bottle top dispenser by the cylinder. Always support both the device and the bottle during transport.

19 RAMAN SPECTROSCOPY

19.1 INTRODUCTION

Raman spectroscopy, like infrared spectroscopy, provides information based on vibrational and rotational transitions of the molecules of a sample. A Raman spectrum, however, is generated by a different process than that which produces an infrared spectrum. Infrared spectroscopy involves a direct absorption of electromagnetic radiation to excite molecules to higher vibrational energy levels (the rotational energy levels of the molecules may increase, decrease, or remain unchanged during this same process). Raman spectroscopy, in contrast, involves inelastic scattering of radiation and a Raman spectrum depicts the amount of this scattered radiation; it is not plotted as a function of frequency of the scattered radiation, but rather, the shift in the frequency of the scattered light (in wavenumbers) compared to its original value. Raman spectroscopy is a category 1 test.

Because infrared spectroscopy and Raman spectroscopy are based on different physical processes, the vibrational transitions that occur with each method are governed by separate selection rules. For infrared absorption, a change in the dipole moment of a molecule must occur for a particular normal mode of vibration, while Raman scattering requires a change in the polarizability of a molecule for a given normal mode.

19.2 APPARATUS AND EQUIPMENT

There are two types of Raman instruments currently available commercially: Dispersive CCD instruments and FT-Raman instruments. The latter are normally accessories to conventional FT-IR instruments and they use the FT-IR optical bench (consisting of the beam splitter, mirror system, and laser) together with a separate sampling chamber and a separate detector for Raman analyses. FT-Raman systems use a near infrared laser for excitation; usually, this consists of a Nd:YAG (neodymium: yttrium aluminum garnet) laser with an 1.06 μm (9434 cm^{-1}) output.

Both dispersive CCD instruments and FT-Raman accessories use filters to remove the Rayleigh scattered light, which typically have intensities hundreds of thousands times as bright as the Raman scattered light. The filters also prevent the detection of low Raman shift peaks, however, and the low frequency limits typically correspond to Raman shifts of 100 cm^{-1} or higher.

19.3 ADVANTAGES AND LIMITATIONS

Some of the advantages of using Raman spectroscopy include: (1) little or no sample preparation is normally required for most samples; (2) this technique can provide data for some materials for which infrared spectroscopy provides little or no information; (3) in certain cases, Raman spectra can be obtained in situ of materials in clear glass or plastic containers; consequently, this method can be used for the rapid screening of a large number of samples in some containers; (4) if a compositional mapping of an inhomogeneous material is desired, a Raman microscope accessory has the means to provide a higher spatial resolution (limited primarily by diffraction of the laser light) than can be achieved with an infrared microscope; (5) compared to an extended-range FT-IR instrument, which can measure absorptions down to 225 cm^{-1}, Raman instruments can collect low frequency data down to 100 cm^{-1} or lower (depending on the Rayleigh filter used), and this low frequency region can be important for characterizing and identifying inorganic compounds, or materials which contain inorganic compounds; and (6) water is a very weak Raman scatterer, so it does not produce spectral interferences and Raman spectra of aqueous solutions can be readily obtained.

The primary limitation of Raman spectroscopy for the analysis of most samples is fluorescence, caused either by the sample itself or by some other components of the sample (these can include some very minor components since fluorescence is a much more efficient process than Raman scattering). Burning of the sample can also occur with materials which are not colored if high laser power levels are involved.

In extreme cases, there are a few colored materials, including explosives, which may ignite from laser heating.

19.4 PROCEDURE

As noted, there is little or no sample preparation normally required with Raman spectroscopy so most solid samples are simply positioned at the focal point of the instrument. Liquid samples are usually held in glass vials. Raman spectra of samples in other transparent containers (constructed of plastic or cellophane) have also been obtained. Whenever samples in such containers are analyzed, scientists should obtain spectra of the empty containers themselves under the similar collection conditions to determine the contribution, if any, of the containers to the spectra.

Collection times for samples will be dictated by the scattering strength of the sample (known as Raman scattering cross sections) and should be long enough to obtain a sufficient signal to noise ratio so that all the relevant sample peaks are readily defined and there is not undue noise in the spectrum. As in the case of infrared spectroscopy, both ends of the spectrum will normally exhibit the highest noise levels due to range limitations of the particular detectors used and the effects of the interference filters used to remove the Rayleigh scattered light.

Results will be recorded in the case notes.

The case file will also contain the following information for each sample Raman printout:

- The number of sample scans
- The date and time of the sample scans
- Unique Identifier of the instrument used (unless the laboratory only has one instrument and that instruments identification is documented in the laboratory's equipment list.)
- The filter used, if any
- The spectral resolution, sample gain, mirror velocity, and aperture used
- The laser wattage used
- The type of detector and beam splitter used
- A peak table for the sample

Any manipulation of the data, such as background corrections, subtractions, etc. will also be recorded on the printout.

19.5 INTERPRETATION

Qualitatively, a Raman spectrum appears similar to an infrared spectrum presented in an absorbance format. By convention, the intensity of a Raman peak is plotted increasing in the upward direction and is depicted using a linear scale (note that this is unlike an infrared spectrum presented in a transmittance format, which consists of a logarithmic scale). Unfortunately, there is no convention on which way the frequency increases on a Raman spectrum, and spectra with frequencies increasing from left to right and from right to left both occur in the scientific literature. It is strongly recommended, however, that spectra be plotted with frequencies increasing from right to left in order to be consistent with infrared data.

The appearance and nature of a Raman spectrum depend very heavily on the frequency of the exciting laser. Consequently, if Raman spectroscopy is used for identification, a comparison to a reference spectrum which has been acquired under conditions identical to that used for the sample should be made (this is not to say that one cannot use Raman search libraries which have been generated on other instruments or collected under different excitation conditions, but the match qualities are not likely to be as high as those obtained from libraries generated on the same instrument).

One important point to remember when analyzing very small samples of crystalline materials is that orientation effects may occur using Raman spectroscopy. Orientation effects arise from the fact that the laser line is very strongly polarized. Orientation effects do not occur when analyzing liquids or other isotropic materials, but if a single crystal (or any material that is anisotropic such as a fiber) is analyzed, the orientation of the crystal or sample relative to the polarization of the excitation radiation may cause preferential scattering for some vibrational modes of the sample. These will affect relative band intensities, so care must be taken in interpreting such variations when analyzing small areas of a sample, a single crystal, or any anisotropic material. In particular, caution must be exercised when interpreting such data collected for two or more exhibits in a comparative analysis. Clarification of differences, if they occur, may require that the scientist run the same sample in several different orientations to verify that they arise from anisotropy and not from actual compositional differences.

19.6 QUALITY ASSURANCE

19.6.1 CALIBRATION

FT-Raman instruments are self-calibrating in that the frequencies determined by the interferometer are based on the frequency of the helium-neon laser of the FT-IR (as distinguished from the laser used for Raman spectroscopy). The frequency of the Nd:YAG Raman laser also does not vary, so there is no regular calibration procedure required. However, the spectrum of a reference material (usually an organic compound which has a rich spectrum with sharp peaks which occur through much of the spectral region) should be run on a regular basis, with the Raman shifts of the peaks indicated on the spectrum. A copy of this spectrum should be maintained in a performance log for the instrument.

19.6.2 MAINTENANCE

The FT-Raman is a robust instrument that requires minimal user maintenance. This maintenance may include routine cleaning, desiccant changes, and part replacement as appropriate. Routine cleaning will include keeping the sampling area clean.

Outside service or any maintenance (excluding routine cleaning) to any part or accessory of the instrument must be recorded in the logbook.

19.6.3 PERFORMANCE VERIFICATION

A series of reference materials will be evaluated on a new instrument or an instrument transferred from another lab. Spectral data of the reference materials will be compared to literature and documentation of these comparisons will be kept with the verification paper work.

19.7 SAFETY

Scientists need to be aware of two aspects of using Raman spectroscopy that may pose potential personal safety hazards: laser exposure and possible ignition of samples.

Direct exposure of laser light can cause severe damage to the retina as the power levels of lasers used for Raman spectroscopy are much greater than those used in a FT-IR instrument (light from a Nd:YAG laser, which is typically used in a FT-Raman instrument, can be 107 times as intense as the helium neon laser used for a FT-IR instrument). When using a laser that is visible to the eye (which would include most of those used with dispersive CCD instruments), viewing the sample with the laser light on is not normally a problem since only diffusely reflected light reaches the eye, and highly reflective samples (such as a mirror) are not usually subject to analysis. The laser light of an FT-Raman instrument, however, cannot be observed so its path and location on the sample are not discernable, and its beam is particularly damaging because of the high power levels involved. Because of this, the laser light of a FT-Raman instrument is not allowed into the sampling chamber when the chamber is open, so there is less of a potential hazard involved with this type of instrument. For either type of system, strict adherence to the safety guidelines outlined in the manufacturer's instruction manual should be maintained.

As already noted in the discussion of limitations, a few samples, including paint pigments and explosives, have ignited from the heat of the laser. Although this is not a problem with the vast majority of samples, it is still a good idea to analyze only a minimal amount of material, particularly if a colored sample is involved.

20 SEM-EDX

20.1 INTRODUCTION

A scanning electron microscope with an energy dispersive x-ray detector (SEM-EDX) is an instrument which can be used like a traditional light microscope to give visual information and can also provide data regarding the elemental composition of a specimen. Rather than using photons as the “illumination” source, the SEM uses electrons emitted from a source at the top of a column, called the “electron gun”. The most common type of electron source is a tungsten filament. The electrons emitted from the filament are focused by a series of electromagnetic “lenses” into a narrow beam directed at a specimen in the SEM sample chamber. The beam rasters, or scans back and forth, in rows across the surface of the specimen. As the beam passes over the specimen, it interacts with it at each focus point in succession. These interactions produce a variety of signals that are collected and processed in real time by various detectors. Imaging is typically the result of secondary electrons emitted from the sample, while elemental data is typically the result of characteristic X-rays emitted from the sample. Both signals can be detected simultaneously as the primary electron beam scans across the specimen. This scanning process allows the SEM-EDX to “map” for each point both the image intensity and a complete elemental spectrum.

20.2 ADVANTAGES AND LIMITATIONS

20.2.1 SEM

As a microscope, SEM provides greater resolution, magnification, and depth of field than light microscopy. An advantage to SEM is that it is essentially a non-destructive method since the sample can be preserved for later examination, although adhesive and/or coating materials may be added in preparing samples for examination.

One limitation is that the SEM produces an image that is monochromatic. This can make landmarks on the sample sometimes difficult to recognize. Another limitation is the possibility that the sample may be subjected to charging, possibly creating damage to the specimen. This can sometimes be avoided or minimized through sample preparation such as carbon coating before analysis (if allowable).

20.2.2 EDX

As a method of elemental analysis, there are several advantages of EDX over other methods. Elements in a sample can be quickly identified. Data may be obtained from a bulk sample, or individual particles within a specimen may be analyzed by spot focusing the electron beam rather than scanning.

EDX also has some limitations. These include the inability to detect elements in trace concentrations or below atomic number 12, the need for conductive coating of some samples, the inability to remove a sample from most embedding materials after analysis, and the discoloration of materials by irradiation. Also, the scientist should keep in mind that although the natural X-ray line width is approximately 2 eV, energy dispersive X-ray spectrometry resolution is generally no better than approximately 140 eV. As a result, there may be an overlap of peaks in the energy dispersive X-ray spectrum of materials containing several elements.

20.3 APPARATUS AND EQUIPMENT

Because the various laboratories have differing models of scanning electron microscopes, the scientist should refer to the user’s manuals for each specific instrument for exact operating procedures. Only general considerations will be described in the following sections. Obtaining optimum results with the instrument requires that the scientist understand how changing various parameters will affect the quality of the data produced. It is essential that along with the user’s manual, the scientist reviews the basics of atomic structure and orbital theory and periodic table of the elements.

20.4 **PROCEDURE**

The specific approach, or procedure performed, is dependent upon the case, or more specifically the evidence sample being analyzed. The scientist should be familiar with the capabilities of both the hardware and software of the SEM/EDX system being used. This will allow the best choice for sample preparation techniques to obtain optimum results.

Before analysis can begin, the sample must be mounted on an appropriate stub or planchett. The size of the sample holder, available stubs, and the sample changer and ultimately the size of the sample changer determine the maximum size for a sample. If the sample needs to be tilted during analysis to examine areas of convoluted topography, this also reduces the maximum size that will fit into the instrument. The typical sample, however, is quite small and can be mounted on an aluminum stub with double sided carbon tape.

In preparing a sample for analysis, the following should be considered:

- The decision to polish the sample or coat it with either carbon or gold will be influenced by whether the goal is to obtain good imaging, measurement of topographical features, or elemental (semi-quantitative) data.
- Forensic samples are almost always “dirty” samples. The circumstances of the case will dictate whether the samples can be cleaned, sliced, polished, or otherwise prepared for analysis. The composition of the sample (conductive or non-conductive, homogeneous or non-homogeneous) will also dictate whether the material may be analyzed with or without coating, polishing, or cross sectioning. Layered samples also present an important consideration when preparing cross sections since the electron beam can penetrate through a surface layer causing the collected data to be from both the surface layer and any underlying layer.
- Once a sample has been prepared and mounted for analysis, the scientist should be aware of any elements in the mounting material that will appear as background interference in the X-ray spectra. A sample of the mounting substrate will be run during the analysis and recorded in the notes.
- SEM/EDX is generally non-destructive and other techniques may be used to examine the sample after this analysis. However, the specific requirements of other analytical procedures should be considered before adding coatings, adhesives or embedding media or otherwise altering the sample.

Images printed from the SEM should be labeled with the case number, item number, scientist initials, date, detector used, and subject of the image (i.e., fiber tips from damage in upper front of shirt). A scale, or relevant markings indicating size, should be included when measurements are reported. The working parameters used to collect the image should be recorded in the notes for the case.

The accelerating voltage of the electron gun should be high enough to cause excitation of characteristic X-rays from expected elements in the sample. Peaks of interest should be labeled with the identified element. Comparison of two samples should be conducted with similar sample preparation and instrumental operating conditions for both samples.

Elemental spectra printed from the EDX should be labeled with the case number, item number, scientist initials, date, and subject analyzed (i.e., questioned blue particle from right pants leg). The working parameters used to collect the spectrum should be recorded in the notes for the case.

20.5 **INTERPRETATION**

Once an X-ray spectrum is collected, a qualitative analysis is performed in order to determine the elements present. The process is straightforward for the peaks of elements present in major amounts and those not overlapping. Misidentifications or omissions of minor components are possible unless a systematic approach to elemental identification which includes consideration of X-ray line families, spectral artifacts, escape peaks, sum peaks, and overlaps is used.

Identification begins with high-energy peaks and major peaks. High-energy peaks are generally less likely to overlap than lower energy peaks. Spectral interpretation then alternates between the identification of major peaks and the associated minor peaks, noting the presence of any lower energy families and their expected relative intensities. Individual asymmetric peaks and inconsistent peak ratios within a family may indicate a peak overlap.

20.6 QUALITY ASSURANCE

With any instrument, proper care, documentation and checks are necessary. How often one performs these procedures, and which procedures are performed, depends upon the types of analysis that are performed. By doing this, one can not only establish how the system is operating, but also determine if there are any potential problems arising. These checks should be monitored at a minimum of once a year.

The procedure checks used with the SEM will be resolution and measurement checks. There are many prepared samples, or test specimens, available through vendors to help with this. Resolution is usually specified as the minimum distance by which two lines or points in the object must be separated before they can be revealed as separate lines or points in the image. Ideal resolution test samples must have high contrast, sharp edges and closely spaced features, and they must be useful at all accelerating voltages. To check SEM resolution one can use a gold, tin, or aluminum-tungsten resolution test specimen or any other comparable specimen. For the measurement check, one can examine a standard specimen or grid of known dimensions and compare the result from the software measurement tool against the known dimensions. It is recommended to check both the resolution of the image and the measurement tool accuracy at different magnifications.

The procedural checks with the EDX consist of spectrum calibration, checks for resolution, sensitivity and spectral artifacts. These checks are performed using standard samples of specific elemental composition such as the X-Checker.

Spectrum calibration sets the correct peak locations (keV value) for elements in a specimen given the location of peaks of a known element. This must be done for each eV channel and time constant on the instrument before that setting is used. The calibration is performed with a sample or standard which gives a strong Kα line below 10 keV; usually an element which gives a peak in the range of 6 to 9 keV, such as Fe, Co, Ni or Cu.

The resolution of the X-ray detector is monitored over time and is used to detect changes in the performance of the detector. This can be accomplished by measuring the width of the Mn Kα peak and ½ height of the peak. To check the low end resolution, one may use a fluorine source such as polytetrafluoroethylene (PTFE, Teflon®) or a fluorine containing mineral such as CaF_2.

Detector windows can be coated with vacuum pump oil or other contaminants that can attenuate the incoming X-ray signal and reduce sensitivity. This can be especially serious when analyzing low energy X-rays. A simple way to monitor this is to perform a peak ratio measurement using a standard that gives high and low energy peaks, such as Mn and Cu. If the Mn Kα peaks becomes larger versus the Cu Lα peak, this indicates a loss in sensitivity for low energy X-rays.

To check for possible artifact peaks from the instrument that may appear in sample data, a spectrum is obtained from a pure carbon sample. This should produce a continuum spectrum with no characteristic peaks besides the very low energy carbon peak. A small rise in the baseline at the lower energies is

Bremsstrählung radiation and is acceptable. There may be a minimal silicon absorption edge peak. If the silicon absorption edge is of significant size this might suggest detector abnormalities or degradation.

As with any instrument, maintenance is a necessary process that allows the instrument to operate efficiently or corrects problems, such as a blown filament. Most of the maintenance of the SEM/EDX is focused around the SEM. Maintenance is of two types: routine and as necessary. Routine maintenance typically consists of checking and changing the rotary pump oil. Refer to the user's manual for the specific instrument for more information on maintenance.

Necessary maintenance is usually due to a problem with the instrument, such as blowing a filament. When this occurs, it is an appropriate time to clean the firing unit and the anode as well. The cleaning of the column or aperture assembly is only necessary if the image resolution deteriorates and cannot be improved by other adjustments.

20.7 **SAFETY**

Appropriate safety precautions should be employed when refilling the liquid nitrogen dewar. When filling the dewar, eye level should be above the funnel. Personal protective equipment including safety goggles, face shields, insulating gloves and longs sleeves should be used when handling liquid nitrogen.

Caution should be exhibited when working with vacuum pumps. Waste oil should be treated as hazardous and should be handled and disposed of appropriately.

The SEM/EDX is a high voltage system. After powering off the system, allow any stored energy to discharge prior to performing any maintenance. All maintenance should be undertaken with caution.

21 SPECTROSCOPE

21.1 INTRODUCTION

A flame test can be performed by presenting the sample to a lightly colored or uncolored flame. Samples containing certain elements will change the color of the flame, the color of which may indicate what elements are present in the sample. Elements emit specific wavelengths of light described as their emission, bright line, or line spectra. These individual emission lines contribute to the observed flame color. A spectroscope separates different wavelengths of light using a diffraction grating or prism. If a scale is included, use of a spectroscope can provide the scientist with information about what element's line spectra are contributing to the observed color. This technique is considered to be a category 2 test.

21.2 APPARATUS AND EQUIPMENT

The WINSCO Model 125 Spectroscope is a replica diffraction grating instrument with a scale to show approximate wavelengths in Angstrom units. In use, a white face reflector is positioned to give a small amount of backlighting to a photographic film scale so that it may be clearly seen against the superimposed image of the spectrum. This instrument has been found to be useful for detection of a number of elements including Ca, Sr, Li, K, Na, Th, Rb, Pb, Cu.

21.3 ADVANTAGES AND LIMITATIONS

Lithium, strontium and calcium all give red or red-orange colors. Observation of the line spectra using a spectroscope allows these three elements to be clearly distinguished. Use of a spectroscope can also help address the possibility of the presence of other elements changing the observed flame color. For example, a small amount of sodium can change the appearance of a red flame observed for a sample with lithium to an orange-red or orange color. The spectroscope can allow the simultaneous detection of both sodium and lithium spectral lines.

Not all elements have strong emissions and may not be observed using flame tests. This method is not quantitative.

The scientist should know from personal experience that the method used to introduce the sample to the flame does not significantly color the flame in and of itself.

21.4 PROCEDURE

When using a spectroscope, eliminate as much external light as possible so that the only light entering the instrument is due to the light source being examined. To view spectra in the laboratory, a source is placed in front of the slit and the adjustable reflector is arranged so that light from some source as an incandescent lamp is directed on the scale sufficient to illuminate it. For detecting various elements, a concentrated solution of the salt is placed in a small beaker. A clean Pyrex test tube, which has been partially filled with cold water, is then dipped into the solution which will adhere to the outside and bottom of the test tube. The test tube is then held in the colorless flame. The salt will vaporize and color the flame, which is examined with the Spectroscope. The purpose of the cold water within the tube is to prevent the glass of the tube from getting so hot as to impart color to the flame.

Another way to color the flame is to dip a nichrome wire in the salt and insert it in the colorless flame. The wire is usually moistened with hydrochloric acid. The salt may be placed in a watch crystal or on a glass plate for this work.

When measuring an emission spectrum, record the wavelength and colors of the individual lines observed through the spectroscope's internal wavelength scale. Because of the thickness of the emission lines, readings should be estimated to the nearest five nanometers.

A method blank will be run prior to running the sample.

21.5 INTERPRETATION

Several sources of spectral line information are available for comparison of observed line spectra. These include, but are not limited to the CRC Handbook of Chemistry and WINSCO's "Spectral Analysis – Emission (Bright Line) Spectra" chart.

Boric acid does not ordinarily color the flame appreciably, but by moistening the boric acid, first with alcohol and then with sulfuric acid, the green flame of boron will be seen. Thallium gives a single, very intense green line, but is poisonous and expensive. Copper gives a broad band spectrum.

Ordinary table salt can or a glass rod heated to incandescence gives the sodium flame, an orange color resulting from strong emissions at 589 and 590 nm.

21.6 QUALITY ASSURANCE

21.6.1 CALIBRATION

The spectroscope does not require calibration.

21.6.2 MAINTENANCE

The spectroscope does not require routine maintenance. Care should be taken to store and handle the instrument in a manner that prevents damage or dust buildup.

21.6.3 PERFORMANCE VERFICATION

Performance of the spectroscope is verified using a reference material, preferably of the same material the scientist is attempting to detect in the sample.

21.7 SAFETY

Care should be taken when working with an open flame. The spectroscope should be used in a functional and operating fume hood as the examination of some samples may result in the evolution of gases. Scientists should be familiar with the material attempted to be detected in the sample and should know any hazards associated with those materials.

22 THIN LAYER CHROMATOGRAPHY

22.1 INTRODUCTION

Thin-layer chromatography (TLC) is a method for separation of chemical mixtures. Compounds are separated from each other based on differences in their interactions between a stationary phase and a mobile phase. For the analysis of seized drugs, TLC is considered a category 2 technique.

22.2 APPARATUS AND EQUIPMENT

- Stationary phase - typically silica gel-coated plates
- Mobile phase
- Developing chamber
- Visualizing agents - sprayed or otherwise applied to stationary phase
- Spray box to contain sprayed visualizing agents
- Capillary tube or micropipette
- UV light source

22.3 ADVANTAGES AND LIMITATIONS

TLC is a separation technique that works well for small samples and can be non-destructive. Preparatory TLC can be used for sample isolation or recovery.

22.4 PROCEDURE

Dry samples are usually extracted or dissolved into a liquid. Liquid samples are either run neat or diluted with a miscible solvent, depending on concentration. A reference sample is prepared from a primary or secondary reference material.

The sample(s) and reference material(s) are spotted equidistant from the bottom of the plate. A capillary tube or micropipette may be used for the spotting. The plate will be spotted with the sample, a procedural blank, the reference material and a mixture of the sample and reference material. The plate will be labeled with the crime lab number and the plate lanes appropriately labeled.

The developing chamber is prepared by adding the appropriate mobile phase system. The liquid level of the mobile phase will be below the initial spot line. The mobile phase is allowed to migrate up the plate, typically to within a few millimeters of the plate's upper edge. The plate is removed and allowed to dry and is then processed with the visualizing agent, if necessary. The plate may be viewed under UV light prior to spraying.

The finished plate is documented for the case file by scanning, photocopying or photographing. The color of the resulting spots will be documented if a method of color copying is not available. If the developed spots are not clearly visible on the scan, photocopy or photograph, notations will be made on the copy to depict the developed plate. In these instances, another scientist will indicate that the notations represent the developed plate by documentation in the case file.

Notes must include composition of the mobile phase and the visualizing agent used. The reference material used for comparison must be documented in such a manner that it is traceable to its original source.

22.5 INTERPRETATION

Retention factor (Rf) values can be affected by several factors such as plate adsorbent uniformity, sample concentration, room temperature and solvent front distance obtained during development (time). Therefore, Rf values are not typically measured and reported. The developed plate is evaluated by visually comparing the distance the spots have migrated on the plate. The sample and reference material should travel the same distance. There must be no separation of the spot resulting from the mixture of the reference material and the sample. There must also be consistency between the reference material and sample in the color of the developed spots.

22.6 QUALITY ASSURANCE

Most mobile phase solutions can be made up and stored indefinitely. The reliability checks for the mobile system and the visualizing agents are performed and documented by the use of positive and negative controls on each plate. Stock containers of the mobile phase solutions will be stored in an appropriate location (e.g., stock room, refrigerator) to maintain their quality.

22.7 SAFETY

UV radiation can be harmful to the eyes and care should be exercised to avoid direct exposure to UV radiation.

Visualizing reagent sprays may be hazardous. Scientists should be familiar with the information listed in the Safety Data Sheet (SDS) for each chemical utilized in this technique.

Good chemical safety practices should be employed when working with reagents. TLC should be performed in a functional fume hood. When the TLC plate has been reviewed and observations recorded, the plate should be disposed of properly and should not be kept as part of the case record.

23 TRACE EVIDENCE RECOVERY

23.1 INTRODUCTION

Locard's Exchange Principle states that whenever two objects come into contact, a transfer of material will occur. Trace evidence that is transferred can be used to associate objects, individuals, or locations. The integrity and significance of trace material as associated evidence relies on proper detection, collection, and preservation. An understanding of the transfer and persistence of trace evidence will assist the examiner in interpreting the significance of the analytical results.

23.2 ADVANTAGES AND LIMITATIONS

The goal of this type of examination is to properly detect, document, collect, and preserve evidence/trace materials from items submitted to the laboratory for examination while taking care to prevent contamination and loss. Proper documentation of evidence location should be made so as to preserve the context of that evidence once it has been removed.

23.3 APPARATUS AND EQUIPMENT

Evidence examination areas should have adequate lighting, easily cleaned surfaces, and a physical environment designed to restrict excessive air currents, static electricity, and general foot traffic. Individual circumstances of each case will dictate additional requirements or modifications. In addition to microscopes and cameras, basic tools and packaging supplies are needed for trace evidence collection.

23.4 PROCEDURE

23.4.1 CASE APPROACH

Microanalysis cases generally involve crimes against people and/or property and typically involve multidisciplinary examinations. Both the type of evidence and the nature of the investigation must be considered when planning what might be a complex multidisciplinary approach to evidence examination.

The evidence item is examined visually and stereomicroscopically. Any dirt, debris, stains, and material adhering to the item need to be documented and may need to be collected. During the examination process, the handling and manipulation of the evidence should be as minimal as possible to best avoid contamination.

Depending on the case scenario, it may be useful to obtain crime scene photos, sketches, or other pertinent documentation from the submitting agency to assist in ~~the~~ understanding the context of the submitted item(s). A review of the medical examiner's report and/or related emergency medical records may also be necessary.

Consider the circumstances of the case, the evidence submitted, the available techniques, and the need to preserve or collect other types of evidence when selecting the methods of collection.

Appropriate protective apparel, such as laboratory coats and disposable gloves, must be worn to prevent contamination from the clothing of the examiner. The apparel must be changed as necessary to avoid contamination or transfer between evidentiary items, locations and personnel.

When working with evidence which may need low level DNA analysis performed, disposable sleeves (or suitable equivalent) and a mask or Plexiglas® shield must be employed. Any sample-handing tools used (such as scissors, forceps, cameras and manipulative areas of microscopes) need to be appropriately cleaned between the preparation of each sample. Refer to the DNA Quality Manual for handling and collection techniques and procedures.

Methods used for detecting trace evidence include, but are not limited to, general visual searches; visual searches assisted by different types of illumination, such as oblique lighting and alternate light sources (UV,IR, laser, high intensity); and visual searches assisted by magnification (microscopes). Record the techniques used for detection, collection and preservation of evidentiary items as well as the location from which they are removed.

Trace evidence recovery and collection techniques used should be the most direct and least intrusive technique or techniques practical. Collection techniques include picking, lifting, scraping and vacuum sweeping. Once collected, trace evidence should be immediately placed into appropriate packaging.

23.4.2 PICKING

Trace evidence may be separated from an item by using clean forceps or other implement. The collected samples should be immediately protected against loss or contamination.

23.4.3 LIFTING

An adhesive-bearing substrate such as a clear tape is repeatedly and firmly patted or rolled over the item causing loosely adhering trace evidence to stick to the tape. The collected lifts are typically placed on transparent backing (e.g., clear plastic sheeting, glass slides, clear plastic or glass petri dishes). This protects against contamination and permits samples to be easily viewed and removed for further comparison. If the tape is overloaded, it will not be able to adhere so be cautious when collecting. In addition, adhesive lifts should not be applied to paper, cardboard, or any other surface that would be difficult to remove the lift from for examination.

Modifications of this method may be used for collection of samples for an instrument specific analysis. For example, a metal stub with carbon tape may be used to recover particles for SEM-EDX analysis. A sample cup with a thin film and mono-adhesive may be used to recover particle for µXRF analysis.

23.4.4 SCRAPING

A clean spatula or similar tool is used to dislodge trace evidence from an item onto a collection surface such as clean paper. The collected trace evidence is immediately packaged in a manner to avoid sample loss. This technique is most often conducted within the laboratory in a controlled environment that reduces the risk of contamination or loss of the trace evidence.

23.4.5 VACUUM SWEEPING

A vacuum cleaner equipped with a filter trap is used to recover trace evidence from an item or area. The filter and its contents should be immediately packaged to avoid sample loss. The appropriate vacuum parts, filter, and trap must be changed and rigorously cleaned between each vacuuming to avoid contamination**.** Consider using this method only after using other collection techniques as it is indiscriminate and may result in the collection of a large amount of extraneous material. Vacuum filters will have a listed pore size. Record this size in the notes if used. Be aware that particles may not be trapped if they are smaller than the filter pore size and such smaller particles may be dispersed in the direction of the vacuum exhaust.

23.4.6 INTERMEDIATE STORAGE/PACKAGING

Paper packets, metal “tins”, ziplock bags or similar containers are good general packaging materials for immediate preservation of trace evidence as it is being collected and prior to examination.

23.4.7 PACKAGING

Once the examination is complete and the trace evidence appropriately packaged, the evidence should either be placed with the original item or packaged separately to be returned to the originating agency. The nature of the items collected will dictate the packaging. A different type of packaging method may be necessary after examination in order to retain the integrity of the original evidence item. The purpose of proper packaging includes:

- To safeguard and protect (prevent loss or contamination) all evidence when it is returned to the submitting agency.
- To prevent tampering of evidence.
- To maintain the integrity of the chain of custody from laboratory to the requesting agency.
- To enable efficient location and recovery of selected evidence at a later date.

The new evidence packaging will have at a minimum: initials, date, lab number, and item number of the examined article. The new packaging may also have specific identifying information or a description.

If the collected material represents a new item, then it must be accounted for by proper marking, sealing, and documentation in the case notes, laboratory report, RFLE, and LIMS as per CLD Operations Manual: 3.4 Evidence Items Produced During Casework and LIMS Manual: 18.06 Itemizing Evidence.

23.4.8 NOTE TAKING

Note taking for trace evidence recovery examination provides a written document of what was received, observed and collected. Some important aspects of documentation include, but are not limited to:

- A brief description of scope of examination in notes if not on the RFLE (e.g., search clothing for glass).
- A descriptive listing of item or items submitted.
- The location of each evidence/trace item found.

Detailed sketches and descriptions of the evidence items may be made. Digital imaging is often used for recording of this information. Refer to the Imaging and Visualization section of this manual for digital imaging procedures.

Specific note taking procedures can be found in the CLD QOM 8. Examination Documentation.

23.5 INTERPRETATION

Data interpretation and report writing for trace evidence recovery examinations primarily consists of accounting for what was observed and collected.

23.6 QUALITY ASSURANCE

In the laboratory, the test primarily used for the presumptive presence of blood is the phenolphthalein test. When a stain is tested for the presumptive presence of blood using phenolphthalein, a known blood reference material, along with a reagent blank, shall be tested prior to testing the stain to verify the reliability of the reagent. When a blood reference material is prepared, it will be spotted onto a suitable substrate, dried, and stored in a suitable container. The container will be labeled with the source of the blood and the date collected. This unique identifier will be logged in the reagent logbook (e.g., WMS 1-28-10). When used in casework, the bench notes will reflect the unique identifier of the blood reference material along with the reagents used.

23.7 SAFETY

Personal protective equipment should be utilized for all evidence handing. A particulate mask should be worn when scraping down evidence as this process can generate airborne dust and blood particles.

Be aware of sharp objects concealed inside clothing while searching evidence. Hands must not be blindly placed into packaging or into evidence clothing pockets.

24 X-RAY FLUORESCENCE SPECTROMETRY

24.1 INTRODUCTION

X-ray fluorescence (XRF) spectrometry is an analytical method for obtaining information about the elemental composition of a sample. It is predominantly nondestructive in nature, although a few samples may be altered by exposure to X-rays. Depending on the specific window material that is used for the XRF detector, the lowest atomic number element that may be detected with this instrument is fluorine (atomic number 9), neon or sodium. The detection limit range varies from parts per million for the heavier elements to high percentage values for the lightest detectable elements. The sensitivity of this instrument is very dependent on the atomic number of the element, and a sensitivity curve starting from the lowest detectable element would show a very steep exponential rise that reaches a slowly rising plateau. This is only a general trend however, as the actual detection limit for a particular element is very dependent on the matrix of the sample and in particular, on the atomic numbers of the other elements in the sample. In general, the higher the atomic number(s) of the other element(s) of the matrix, the less sensitive the technique. The detection limits are also quite dependent on the mode of excitation, that is, on the intensities and the energy distribution of the X-rays used to excite the sample, and for the lightest elements, whether the analysis occurs in a vacuum or not.

24.2 APPARATUS AND EQUIPMENT

There are two main types of XRF spectrometers manufactured commercially: conventional spectrometers and micro-XRF systems. Conventional spectrometers are usually equipped with filters or secondary targets, a rotating sample carousel that permits many samples to be loaded into the sample compartment for sequential analysis, and a relatively large compartment that allows a fairly wide range of sample sizes. Micro-XRF systems use only primary excitation to analyze small spot sizes (usually around 25 - 100 microns [0.025 - 0.1 mm] in diameter) and can be used to map the distribution of elements in an inhomogeneous sample or to analyze smaller samples than possible using a conventional instrument.

Each type of XRF instrument uses primary X-rays generated from an X-ray tube, which usually has a rhodium metal target. A stream of electrons impinges on the target, creating two types of X-rays: a continuum and X-rays characteristic of rhodium, consisting of L lines and K lines (both of these are only produced, however, after the electrons have enough energy to ionize the L shell electrons and the K shell electrons of rhodium, respectively). The energy and current of the electrons are set by the user, and this determines the maximum energy that X-rays from the tube can have and their intensities, respectively.

In a micro-XRF system, the primary X-rays from the tube serve as the only source of excitation energy, whereas a conventional instrument may also have several secondary targets that can be used. Secondary targets produce primarily K lines of the element comprising the target and typical target materials may include silicon, titanium, iron, germanium, molybdenum, tin and gadolinium. Since the $K\alpha$ lines are approximately six times as intense as the $K\beta$ lines, secondary targets essentially serve to produce monochromatic X-rays for excitation.

A third configuration is where the XRF tube is mounted in an SEM chamber which will facilitate rapid analysis of small particles, extending the range of elemental identification to heavier atomic number elements. The XRF operating software is used to interface within the SEM platform or to run as an independent program for both EDX and XRF spectral generation. The XRF tube has a voltage from 0-50 kV, an emission current from 0-1.0 mA, a beryllium window, and a poly-capillary focusing optic.

24.3 ADVANTAGES AND LIMITATIONS

In comparison to other elemental analysis techniques, the main advantages of XRF spectrometry are the wide range of elements that can be detected, the ability to simultaneously analyze all of these elements, the wide variety of samples that can be analyzed with little or no sample preparation, the nondestructive nature of the analysis for the vast majority of samples, and the fact that under certain conditions, quantitative analyses can be performed (although for most samples of forensic interest—which are usually small, irregular in morphology, and occur in widely-varying matrices—quantitative analyses are precluded, although qualitative analyses and comparisons are still possible). For most samples, there is minimal sample preparation, and fairly large objects can be analyzed intact. Using a micro-XRF system, it is possible to obtain an elemental distribution map for inhomogeneous materials. For the analysis of heavier elements, there is no need to use a vacuum.

The limitations of XRF spectrometry include the fact that the lowest elements of the periodic table cannot be detected, the sensitivity of the technique is quite low for the lower detectable elements and for these, the analysis must be conducted under a vacuum, and the fact that XRF analyses are generally not quantitative. A particular concern for forensic examinations involving comparisons is that the ratios of X-ray peak intensities for small samples with irregular morphologies (which constitute the majority of trace cases) may vary, depending on how the analysis is conducted; extreme caution must thus be exercised when interpreting such data to determine comparative identity.

In comparison to an energy dispersive X-ray analysis conducted with a scanning electron microscope (SEM/EDX), XRF analyses often do not require a vacuum and the sensitivity is far greater for heavier elements (particularly when using secondary targets). There may also be less ambiguity involved in assigning peaks since two series can be detected for many elements (for example, the K and L lines of heavy elements such as barium and tin can all be observed with an XRF instrument while only the L lines are seen using SEM/EDX). This is particularly useful feature when analyzing materials containing more than one detectable element as overlapping peaks can be a problem using both types of instruments. An SEM/EDX analysis, on the other hand, is more sensitive for the lowest detectable elements, and much smaller areas or smaller samples can be analyzed. The spatial resolution of an analysis is also much greater than that permitted by a micro-XRF analysis.

24.4 PROCEDURE

Sample preparation for XRF analysis is generally minimal. Powders and liquids can be analyzed using disposable sample cups with a thin Mylar (polyester) sheet window. If necessary, the chamber may have to be purged with helium for a liquid suspected of containing one or more of the lighter elements, although the analysis time should be kept to a minimum in such cases. The above precautions do not preclude the analysis of unknown materials using XRF spectrometry, but scientists should certainly have some idea of the nature of the unknown material before an XRF analysis is performed

When analyzing samples for the presence of the lightest detectable elements (and particular for sodium or magnesium), a vacuum or helium purge should be used, and samples should be analyzed without a window or substrate. One means to accomplish this is to place a strip of adhesive tape sticky side down across the sample cup holder and press the sample against the bottom of the tape so that it is securely fastened to the tape.

In cases where a sample is analyzed in a disposable cup with a Mylar window, a tape strip, or with some other substance, a blank consisting of an empty cup, a strip of tape, or other substrate should be run under similar conditions to those used for the sample. The sample and blank spectra should be overlaid or otherwise compared so that peaks of the sample can be clearly distinguished from those observed for the blank.

Total collection times are dictated by the sample, its size, the desired signal to noise ratio, and the flux of X-rays reaching the detector (as well as other practical considerations already mentioned). The flux can be controlled by setting the excitation conditions, especially the current going to the X-ray tube. This should be set so that an optimal flux is reaching the detector, that is, one that is sufficiently high so as not to saturate the detector (which lead to high dead times). A dead time from 20 to 30% is considered optimal; using secondary targets or small samples, even this value may not be reached even if the highest available current is used.

For the iXRF mounted in a SEM, set the SEM beam current to 1 keV, working distance at 25 mm, Z at approximately 20 mm and image the sample using the secondary electron imaging mode. Turn on the current to the electron beam. Locate and focus the glass coverslip and slowly change Z to approximately 15 mm. Turn on the XRF power supply. Open the X-ray shutter on the controller unit. At low magnification of less than 100X, slowly raise Z to approximately 13 mm while looking for a “ghost-like” image of the XRF beam on the glass. Overlay a measurement tool on the image using a point or cross that can be adjusted in the X and Y direction and superimpose the point or cross over the image of the beam. Move the particle to the beam and set analytical parameters for XRF analysis using the SEM system. In the spectrum acquisition menu, set the desired acquisition time and spectrum range. Collect spectral data as in normal EDX operation.

Sample preparation for the SEM/XRF system may include beryllium stubs and/or a fabricated Lexan™ mount for sample placement instead of the adhesive carbon mounting tabs typically used for SEM samples as they contain a variety of trace element contaminates and are not recommended for use. Using a forceps place samples on the beryllium or Lexan™ mount with a small piece of cover glass adjacent to the sample. The cover glass surface is used to image and locate the XRF beam. Place the sample mount directly on top of the SEM sample carousel and pump down the chamber.

Results will be recorded in the case notes.

The case file will also contain the following information for each sample XRF printout:

- Voltage and current used
- The date and time of the experiment
- Unique Identifier of the instrument used (unless the laboratory only has one instrument and that instruments identification is documented in the laboratory’s equipment list.)
- Dead time, if available
- The filter or secondary target used, if any
- The collection duration (live time or counts as appropriate)
- The element identification indicated for all significant peaks
- Identifier of any overlaid background or comparison spectra (file name or description)

Any manipulation of the data, such as background corrections, subtractions, etc. will also be recorded on the printout.

24.5 **INTERPRETATION**

Although each X-ray series observed using an XRF instrument is composed of several closely spaced lines, most of these are not completely resolved by the Si(Li) detector or Silicon Drift Detector (SSD). What is observed are some very characteristic patterns consisting of relatively broad peaks. The K series usually consists of two peaks with the lower energy peak (known as $K\alpha$) having a relative intensity approximately six times as intense as the higher energy peak ($K\beta$).

For a few of the heavier elements (roughly molybdenum, atomic number 42, through barium, atomic number 56) for which K lines are observed, both Kα and Kβ begin to be resolved into two peaks. L series lines consist of two main peaks of roughly equal intensities plus smaller satellite peaks between the two and on either side. M series lines are not resolved at all and only a single peak is observed.

There are no elements for which K, L and M series peaks all occur between 1 and 40 KeV. From the lightest detectable element up to bromine (atomic number 35), only the K lines are observed and for the lightest of these, the two peaks of the K lines merge into a single peak. For elements bromine (atomic number 35) through promethium (atomic number 61), both L lines and K lines are observed, although for the lightest of these, the L lines merge into a single peak. For almost all of the heavier elements, both M and L lines are observed. The intensity patterns for K, L and M series peaks are very important features for scientists to remember since they can aid in assignment of peaks and help distinguish them from artifacts.

24.6 **QUALITY ASSURANCE**

There are two critical areas where scientists must exercise extreme caution in order to protect the XRF instrument: (1) Maintaining the Si-Li detector at liquid nitrogen temperatures (if the instrument has the type of detector where this is necessary), and above all, insuring that the detector is cold when it is turned on. If the detector is not at liquid nitrogen temperatures when the high voltage is applied across the detector crystal, the lithium ions will be removed from their sites, destroying the detector and resulting in a very costly repair bill. The SSDs are room temperature detectors and thus do not require liquid nitrogen for cooling. (2) The window to the detector is extremely thin and fragile. Scientists must ensure at all times that no object is placed near the detector. When objects are placed on a revolving sample carousel, scientists should insure that such objects are secured and will not protrude below the intended analysis position when it is rotated.

XRF instruments otherwise require little maintenance. The X-ray tube coolant level should be maintained. In most instruments the X-ray tube is a sealed unit and does not require coolant maintenance. Instruments may have a safety switch that prevents the X-ray tube from operating when the flow of the coolant is below a certain level, but this switch should not be relied on.

For the Bruker microXRF instruments with SSDs, the recommended quality check is to calibrate the instrument each day of use using the manufacturer provided zirconium reference material. The calibration is achieved by setting the spectrometer to calibrate to the zirconium Kα peak at energy of 15.775 keV. Successful calibration is observed by the scientist and documented in the instrument logbook.

Instrument function and X-ray energy monitoring for the Jordan Valley XRF spectrometer will be conducted each day this instrument is used for the analysis of evidence. This is done as part of the X-ray tube warm-up procedure using the Jordan Valley calibration software. Three internal calibration standards, consisting of aluminum, copper and tin are analyzed, and the K lines of these elements (as indicated by their positions on the monitor display) are shown superimposed on the X-ray spectra. Scientists then assess the correspondence between the peaks of the K lines of the three elements and these cursor positions. If the known K lines of aluminum, copper and tin are not contained within the peaks of the test run, a re-calibration may need to be performed using procedures recommended by the instrument manufacturer. A hard copy of each monitoring spectrum will be dated, initialed by the scientist and kept in a three-ring binder.

The iXRF in the SEM is acting only as a source of X-rays for excitation for the SEM, thus the quality assurance procedures for this instrument are covered in the SEM/EDX quality assurance section of this manual.

It is a good practice to record when the liquid nitrogen dewar has been filled (where applicable).

A series of samples spanning atomic numbers from the lower range to the higher range should be analyzed to verify that new instruments are functioning appropriately. This data should be maintained in the instrument log books.

Verification that the safety interlock is operational must be done each day of use (per WAC 246-227-130). This will be recorded in the instrument logbook.

24.7 SAFETY

XRF instruments are surrounded with lead shielding and equipped with safety switches which prevent the X-ray tube from being turned on if one of the access panels is removed.

Appropriate safety precautions should be employed when refilling the liquid nitrogen dewar. Personal protective equipment including safety goggles, face shields, insulating gloves and long sleeves should be used when handling liquid nitrogen.

The exit window of the X-ray tube is covered with a think beryllium window and is highly toxic. If breakage of the window occurs, avoid inhaling the particles and do not allow them to come in contact with skin or clothing.

PART THREE: DRUG ANALYSIS

25 SEIZED DRUGS

25.1 INTRODUCTION

Seized drug evidence generally contains seized drugs, adulterants, diluents, and impurities. Analysis of seized drugs in the Washington State Patrol Crime Laboratory Division typically involves the qualitative examination of suspected drug evidence to determine if the material contains a controlled substance, designer drug, or other drugs of known forensic interest. Controlled substances will include chemicals listed in the Federal and/or Washington State Uniform Controlled Substances Acts on the date of the offense. These requirements will also pertain to substances determined to be controlled substance precursors and legend drugs as defined by the Revised Code of Washington (RCW) or the Washington Administrative Code (WAC). The following analytical approach is congruent with the current version of ASTM E2329 – Standard Practice for Identification of Seized Drugs.

25.2 ADVANTAGES AND LIMITATIONS

Drug evidence is often more homogeneous than other types of evidence, and processes for the identification of seized drugs are often quite rapid.

Due to the variable nature of the evidence received in seized drug casework, a single approach or set of methods cannot adequately address all possible contingencies. The technical procedures in this document are therefore understood to be flexible to the requirements of each particular case.

This service is limited to the qualitative analysis of suspected seized drugs. The identification of other compounds known to be associated with controlled substances may be performed. Comprehensive qualitative characterization of materials for investigative purposes is covered by other types of service (see General Chemical Analysis chapter). Substances which are considered ‘emerging drugs of abuse’ may be reported with qualifying language based on the availability of validated external spectra.

25.3 APPARATUS AND EQUIPMENT

The analysis of suspected seized drugs utilizes a combination of analytical techniques. Part Two of this manual discusses in detail the Instrumentation and Techniques which are available to the scientist for the analysis of seized drugs.

25.4 PROCEDURE

Case Approach

In the course of an analysis, the forensic scientist is expected to make observations, generate data, and evaluate this information in order to determine a course of action. Forensic scientists must use their scientific knowledge and experience to determine the appropriate course of analysis in each case.

It is the responsibility of the forensic scientist to evaluate evidence received and analyze selected items for the presence of seized drugs. The specific analytical techniques to be used in an analysis should be chosen by the scientist to be sufficiently sensitive and specific for the particular substance(s) or class(es) of substance involved. It is expected that an appropriate analytical scheme effectively results in identification with high scientific certainty. The conclusive identification of a seized drug is accomplished by the use of at least two uncorrelated analytical techniques. At least one of these techniques must provide molecular structural data about the substance. This must be accompanied by additional testing using another analytical method or methods. These additional methods need not provide molecular structural data, but should be sufficiently specific for the analyte in question as to provide a meaningful confirmation of the results of other techniques.

For the purposes of this document, techniques for the analysis of seized drug samples may be broken down into two categories based on their discriminating power. A Category 1 technique has reviewable data and provides molecular structural data about the substance. Category 2 techniques are other discriminating techniques, but do not fit into Category 1 either because they lack reviewable data or they do not provide molecular structural data. Some examples of reviewable data include printed spectra, chromatograms, and photographs or photocopies of TLC plates. Recording of detailed descriptions of morphological characteristics will be allowed for marijuana. Table 1 lists examples of some techniques:

Table 1: Categories of Analytical Techniques

Category 1	Category 2
Infrared Spectroscopy	Capillary Electrophoresis
Gas Chromatography-Mass Spectrometry	Gas Chromatography
Nuclear Magnetic Resonance Spectroscopy	Ion Mobility Spectrometry
Raman Spectroscopy	Liquid Chromatography
Liquid Chromatography-Mass Spectrometry	Microcrystalline/Microchemical Tests
Gas Chromatography-Infrared Spectroscopy	Pharmaceutical Identifiers
	Thin Layer Chromatography
	Spectroscope

A Category 1 technique will be used for the identification of a seized drug. At least one other technique (from either Category 1 or 2) must also be used. This combination must identify the specific substance present and must preclude a false positive identification. When sample size allows, the second technique should be applied on a separate sampling, for quality assurance reasons. Gas chromatography-mass spectrometry (GC-MS) is regarded as a single test for the identification of seized drugs. Chemical identification of adulterants, diluents, or impurities must be based on a technique, or combination of techniques, that are of sufficient discriminating power to allow for the identification of the chemical in question. GC-MS may be considered as separate tests for non-controlled adulterants and diluents, provided that the results from each are used. If GC-MS is used for both required tests in the identification of seized drugs, the use of a different column functionality and/or a chemical derivatization of the sample is required for the second test. Qualified conclusions may be reported at the discretion of the analyst provided the report clearly states the substance was not conclusively identified. An example of a qualified conclusion could state “The presence of methamphetamine was indicated; however, additional testing will be required for conclusive identification.”

Analysis of Marijuana Exhibits
Marijuana exhibits are an exception to the requirements outlined above for the identification of seized drugs. The analytical requirements for the analysis of marijuana exhibits will be covered in the Marijuana section of this manual.

Weighing/Counting
The amount of material received in each exhibit analyzed will be documented and reported. For most solid exhibits, the material will be weighed, without packaging whenever practical. When the mass is reported it may be truncated by dropping the least significant figure(s). If packaging is included in recorded weights, this will be noted. If gross weights are used in the final report, the report will indicate what packaging is included. The total weight of multiple exhibits may be estimated based on the measured weight of a smaller number of similar items. Weights of residues and samples that cannot be accurately weighed may be estimated based on the sample's appearance. If estimated weights or volumes are included in the report, they will be specified as such. Volumes of liquids should be measured or estimated as is practical. Tablets, capsules, injection vials and other such items should be counted when practical, or a total count should be estimated, or a weight of the items may be obtained.

Measurement uncertainty reporting requirements are described in the Marijuana and Measurement Uncertainty sections this manual.

Methods of estimating any weights or volumes (other than by visual observation) will be recorded in the case notes. Solid or liquid residues that cannot be practically or accurately weighed or measured may be reported as "a residue", "a trace", "much less than 0.1 gram", or similar language. These expressions need not be specified as an estimated quantity in the report.

Sample Selection
Sample selection is the practice of selecting a sample(s) of the whole based upon training and experience to draw conclusions and report only on the sample(s) tested.

The case notes will reflect what was selected for analysis. It is desirable that different portions be selected from the exhibit for analysis by different techniques. Exhibits containing a small amount of material may not allow multiple portions to be taken. When this occurs, multiple examinations may be conducted on a single portion of an exhibit. At least half of each exhibit must be preserved for possible future analysis unless prior arrangements have been made with the submitting agency or the prosecuting attorney. Refer to the QOM ~~9.~~ Limited Sample for documentation requirements.

Data Collection
Data collected in the course of an analysis may include printed instrumental data, written measurements or observations, or pictorial reproductions. Any conclusions drawn by the forensic scientist about a sample will rely on this body of information. Those who examine case files and case data in the future (in technical review, during court proceedings, and in the course of subsequent analyses) will also rely on being able to interpret the data that was collected, and to understand clearly how a sample was analyzed. Great care should be taken that observations are accurately and clearly recorded and that instrumental data, photographs, etc. are of good quality.

For reported substances, all spectral (e.g., mass, infrared), chromatographic (retention times) and other instrumental data will be compared to appropriate reference data. The comparison will be documented in the case notes by including a hard copy of the reference data. Instrumental parameters for reference data may be documented in the case notes or the case record.

25.5 INTERPRETATION AND REPORTING

The written report should accurately and clearly convey to the reader the items received and the result of the analytical work done on the evidence submitted for examination. The report language should be accurate, clear, and understandable. Any limitations to conclusions reached should be clearly specified.

Cases in which seized drugs are not identified will be reported to indicate such using one of the following options:

- No controlled substances or known drugs of abuse were identified.
- No controlled substances or known drugs of forensic interest were identified.

The report will reflect if the sample was consumed in the course of the analysis in cases in which limited amounts of sample have necessitated pre-approved sample consumption. See the Seized Drugs Training Manual for more information regarding specific substances and testing limitations which would impact reported results.

With compounds controlled by a chemical class in either the RCW or WAC, reports will use the format: "'Item' contains 'common or trade name', a 'class name'". The chemical name may be added in parenthesis after the common or trade name. The chemical name used will be listed in the Names and Verification References of Designer Drugs document on the Portal; if the chemical name is not in that document, the name from the supplier's information sheet will be used.

With compounds that are not controlled but are structurally similar to a controlled substance, this similarity may be reported. The phrase "structurally similar to" and the name of the controlled substance in question will be used. The term "analog" has legal significance and will not be used.

- For example: "Item 101 contains AM-2201 (1-(5-fluoropentyl)-3-(1-naphthoyl)indole), a naphthoylindole."
- For example: "Item 102 contains 2,3-methylenedioxyamphetamine, which is structurally similar to 3,4-methylenedioxyamphetamine".

When there is either literature information but no verification reference material available or there is reference material available that is not verifiable, reports will use the format "'Item' was consistent with 'name', but could not be conclusively identified because a verified reference material was not available."

If a specific isomer is controlled in the RCW or WAC but is not determined analytically, reports will use the format "'Item' contains 'name'. The optical isomer was not identified." or "The specific isomer was not identified."

Statements concerning legal control (controlled or non-controlled, or controlled in the WAC but not in the RCW, etc.) will not be routinely reported.

Exceptions to any format may be approved by the supervisor on an individual case basis.

25.6 QUALITY ASSURANCE

Instrument calibration and maintenance will be performed on a regular basis, as outlined in each instruments' technical procedures. Calibration reports and maintenance are documented and can be found with the respective instruments or in an electronic logbook.

Quality check results are to be evaluated. If the results are not adequate for the instrument, equipment or technique, then the instrument, equipment or technique is not to be used until appropriate action steps are taken to correct the problem. A quality check that yields satisfactory results deems the instrument, equipment or technique appropriate for use.

It is essential to ensure that data collected by any analytical method is not subject to confusion or misinterpretation resulting from contamination of the case sample.

Practices to be used to prevent contamination of evidence must be employed at all times. Work areas should be kept orderly and cleaned regularly. No more than one item at a time of controlled substance evidence should be open in the scientist's work area. Reusable utensils or glassware should be thoroughly cleaned prior to each use. Disposable glassware and utensils will be used only once and then discarded. Appropriate blanks, including blanks of extraction, must be performed for all analyses in accordance with the technical procedures. Auto-sampler vials should be clearly marked and securely closed.

25.7 SAFETY

Safety precautions for each analytical technique are outlined in the appropriate technical procedure manuals. The use of gloves and other personal protective equipment is highly recommended when handling seized drugs evidence.

25.8 DRUG REFERENCE MATERIALS

25.8.1 INTRODUCTION

Each laboratory may retain drugs in a laboratory drug reference collection as necessary for reference materials, reagent testing, training, proficiency testing and customer and public education.

Each laboratory's Materials Analysis Section supervisor, together with the Laboratory Manager, will be responsible for oversight of the purchase, documentation, and distribution of drug reference materials as needed. The purchase of controlled substances will follow Drug Enforcement Administration (DEA) and Washington State Board of Pharmacy guidelines.

The provisions of this section do not apply to dilute laboratory working solutions of drug reference materials unless specified.

Definitions

Controlled Substance
A controlled substance refers to any drug (compound) listed in the Revised Code of Washington (RCW) Chapter 69.50 (Uniform Controlled Substance Act) or the Code of Federal Regulations Title 21 Part 1308 as a Schedule I, II, III, IV or V controlled substance.

Drug [as defined by 21 U.S.C. 321 (g)(1)]

- Articles recognized as a drug in the official United States pharmacopoeia/national formulary or the official homeopathic pharmacopoeia of the United States, or any supplement to them;
- Articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in individuals or animals;
- Articles (other than food) intended to affect the structure or any function of the body of individuals or animals; and
- Articles intended for use as a component of any article specified in (1), (2), or (3) of this subsection. The term does not include devices or their components, parts, or accessories.

Drug Reference Collection
All primary reference materials, secondary reference materials, training samples and paraphernalia retained by the Crime Laboratory. This does not include known or suspected seized drugs received as evidentiary materials for analysis or dilute laboratory working solutions of drug reference materials.

Inventory (as defined in 21 CFR 1300.01)
All factory and branch stocks in finished form of a basic class of controlled substance manufactured or otherwise acquired by a registrant, whether in bulk, commercial containers, or contained in pharmaceutical preparations in the possession of the registrant.

Legend Drug
Any drugs which are required by state law or regulation of the state board of pharmacy to be dispensed on prescription only or are restricted to use by practitioners only.

LIMSRef
The Drug Reference Material Management System used to store information related to the drug reference collection.

Marijuana Product
Any pre-packaged item of marijuana, marijuana-infused product, marijuana concentrate or cannabis health and beauty aid acquired as a drug reference material.

Paraphernalia (as defined in RCW 69.50.102)
All equipment, products, and materials of any kind which are used, intended for use, or designed for use in planting, propagating, cultivating, growing, harvesting, manufacturing, compounding, converting, producing, processing, preparing, testing, analyzing, packaging, repackaging, storing, containing, concealing, injecting, ingesting, inhaling, or otherwise introducing into the human body a controlled substance.

For the purpose of the WSP Crime Laboratories, paraphernalia will include items intended for making, using, delivering and concealing a seized drug and will not include standard forensic and/or analytical laboratory supplies and equipment such as balances, evidence packaging material, instrumentation, etc.

Primary Drug Reference Material
A commercially purchased compound that is traceable back to a manufacturer.

Secondary Drug Reference Material
A laboratory produced or case work sample that has been analytically verified to a primary reference material or to the appropriate published literature.

Training Material
A laboratory produced sample, case work sample or sample slated for destruction obtained from a law enforcement agency.

25.8.2 PROCEDURE

Purchase of Marijuana from Retail Stores

Scientists may find it necessary to purchase marijuana products from licensed retail stores in order to obtain relevant marijuana products for research and method validation. If the retail store does not accept a state purchase card, the employee may use their personal cash and submit for reimbursement. For all purchases of marijuana products, the following shall apply;

- All purchases shall be pre-approved in writing (email, IOC, etc.) by the scientist's supervisor or lab manager. The request for purchase shall list the type of item to be purchased and the purpose.
- The retail store shall be licensed by the Liquor and Cannabis Board.
- All purchases at retail shops will be witnessed by a CLD employee. Both the purchasing scientist and the witness shall sign and date the purchase receipt provided by the retail store.
- Purchases shall not exceed $200 in one transaction.
- Purchases shall not exceed $600 per year at any one retail store.
- Purchased marijuana products will be regarded as primary drug reference materials, promptly recorded in LIMSRef, and secured accordingly.
- Documentation for reimbursements shall include the written approval, purchase receipt with purchaser and witness signatures and date, and a printed copy of the LIMSRef entry demonstrating the purchased product was recorded in the drug database.

Receipt of Drug Reference Material

Controlled Substances and legend drugs

Upon receipt, any controlled substance(s), legend drug(s), marijuana product and paraphernalia will be recorded in LIMSRef following the procedures outline in the LIMSRef Operations Manual. Proficiency test samples from the same proficiency testing batch may be combined into one sample for entry into the drug reference collection. Additional information regarding proficiency test samples is outlined in QOM 17.3.2. The procedure for obtaining secondary drug reference materials from casework is outlined in QOM 14.3.

Other substances

All drugs and chemical substances that are possessed by a laboratory, used as reference materials, and not classified as controlled substances or legend drugs will be logged into LIMSRef or the laboratory's chemical inventory as appropriate. For substances included in the chemical inventory, the following information will be documented:

- Name of the chemical
- Amount received
- Storage location

Manufacture of Drugs

Drugs manufactured in the laboratory will be added to the drug reference collection following the procedure described above. Intermediates or by-products produced during the manufacturing process which may be used as reference materials or training samples will be added to the drug reference collection. Additionally, the following information will be recorded in LIMSRef:

- The date of manufacture
- The analyst responsible for the manufacture
- A note regarding any inventoried precursors used in the manufacturing of the drug which is being entered into the reference collection

Identification of Reference Material

Each bottle, vial, bag, piece of paraphernalia or other sample enclosure containing an item from the laboratory's drug reference collection will be clearly marked with a unique internal control number.

Storage of Reference Material

All primary and secondary drug reference materials and training samples will be stored in a locked cabinet, drawer, room, or other secure area at all times when not under the direct control of authorized personnel. Access will be limited to scientists authorized to analyze seized drugs, seized drugs analysis trainees, laboratory management, and designated auditors. Training samples or paraphernalia used for display purposes should not be accessible to the general public.

Verification of Reference Material

All controlled substances, legend drugs and non-controlled substances used as reference materials for comparison to casework, reagent verification and instrument verification will be verified prior to use.

Verification will consist of analysis by instrumentation which will provide reviewable molecular structural data. The instrument selection for verification will be appropriate for confirming the form of the material including salt forms, esters, etc., as necessary.

Verification must be independent from the supplier to demonstrate that the reference material chemical identity is correct. The verification data will be compared to a published reference from a reliable source that was published by a reputable scientific body and is accepted in the scientific community. Journal articles should include structural elucidation coupled with MS and/or IR data. Peer reviewed publications in which structural elucidation is not included will be evaluated on a case-by-case basis for use in verification of reference materials by the Technical Lead. In addition to reviewed scientific journals, the following resources have been approved for the verification of reference materials:

- NIST Mass Spectral Libraries
- Aldrich Library of FT-IR Spectra
- IDDA
- Clarke's
- ForensicDB
- CND Analytical
- Wiley Mass Spectral Libraries (including Mass Spectra of Designer Drugs)
- SWGDRUG Monographs
- SWGDRUG Libraries

A copy of the verification data and a copy of the published reference or a notation as to the source of the published reference will be maintained in LIMSRef.

The date of verification will be entered into LIMSRef or chemical inventory.

Materials which do not pass the verification process will not be entered into the system as a verified reference and will be destroyed, returned to the vendor, or undergo other appropriate actions to prevent them from being used as verified reference materials.

Subsequent usage of a reference material will be assessed to ensure that the material still returns the same result as when initially verified. Any deterioration or changes in a reference must be noted and other scientists in the lab made aware of the issues. If a reference is no longer suitable for use as a reference, it may be destroyed or converted to a training sample, if appropriate.

A primary reference material (whether controlled, a legend drug, or non-controlled) having an expiration date can be used as a reference material for qualitative analysis beyond the expiration date if the expired reference material is verified after the expiration date, even if it has previously been verified. The verification data will be reviewed to confirm that the reference material is suitable for continued use.

Use of the Drug Reference Collection

Whenever a portion of a controlled substance or legend drug is removed, the usage will be documented in LIMSRef according to the procedures described in the LIMS Ref Operations Manual.

The usage of materials other than controlled substances or legend drugs which are inventoried in LIMSRef may be documented in LIMSRef.

Depletion and Destruction of a Drug Reference Item

Depleted items from the drug reference collection will be witnessed and documented in LIMSRef according to the procedures described in the LIMSRef Operations Manual.

When no longer of use, items from the drug reference collection will be destroyed. The destruction information will be documented in LIMSRef. The item to be destroyed will be sealed in a paper bag, evidence envelope or other appropriate package and will be assigned a WSP Property/Evidence number. The WSP Property/Evidence barcode stickers can be obtained from a WSP District PEC or purchased from WSP Supply. A WSP Property/Evidence Report (form 3000-110-096) will be completed and the item to be destroyed will be signed over to a WSP District PEC. Copies of the WSP Property/Evidence Report that contain the signatures of the releasing scientist and WSP District PEC will be scanned and uploaded to LIMSRef.

DEA Form 222

The DEA Form 222 is required for each distribution of a Schedule I or II drug except those acquired by laboratory manufacture or from case evidence slated for destruction.

Only persons listed on the registration or designated power of attorney may complete the DEA Form 222

Power of Attorney requirements are detailed in CFR 1305.05.

Forms must be stored in a secure location. Procedures for lost or stolen forms are detailed in CFR 1305.16.

DEA Registration and WA Department of Health Licenses

Each laboratory's DEA Registration and WA Department of Health license and DEA Forms 222 shall be kept in secure locations within the laboratory.

Ordering Schedule I or II controlled substances

The DEA Form 222 will be completed in ink following all the instructions on the form or detailed in CFR 1305.13.

Errors on the form may necessitate voiding the form. All voided forms will be maintained for a minimum of two years.

When the form is properly filled out and signed, copy 3 will be kept by the purchaser and copies 1 & 2 will be forwarded to the supplier with the appropriate carbon sheet in place.

Transfer of Drug Reference Collection Items

Transfers of bulk controlled substances or legend drugs transferred between laboratories in the Crime Laboratory Division will ~~be~~ follow the procedures described in the LIMSRef Operations Manual. If the sample being transferred is a Schedule I or II substance initially acquired with a DEA Form 222, a Form 222 will be required in addition to the Drug Transfer Record.

Dilute solutions may be transferred between labs as described in the LIMSRef Operations Manual.

Drug Reference Collection Audit

An annual audit of the inventory of the complete drug reference collection will be performed by the Quality Process Manager or designee. The annual audit, referred to as an inventory in CFR 1304.11, will be conducted using reports generated by LIMSRef that include:

- A list of all Schedule I & II substances
- A list of all Schedule III, IV, & V substances
- A list of all legend drugs, non-controlled drugs and paraphernalia

The annual audit will also include a review of the following records:

- Completed and/or voided DEA Forms 222
- Transfer records
- Records of depletion and/or destruction
- Usage records

Every other year the audit will include the current gross weight of each primary reference material, secondary reference material and training sample in the drug reference collection. Items of paraphernalia in the drug reference collection do not need to be weighed.

The records of this audit, which will include the DEA Registration Number, will be maintained in LIMSRef. An IOC detailing the audit and any discrepancies will be forwarded to SAS.

25.9 MARIJUANA

25.9.1 INTRODUCTION

The definition of marijuana changed on December 6, 2012, with the implementation of I-502 to state “marijuana” or “marihuana” means all parts of the plant *Cannabis* whether growing or not, with a THC concentration greater than 0.3 percent on a dry weight basis. The law further states “THC concentration” means percent of delta-9-tetrahydrocannabinol content per dry weight of a part of the plant *Cannabis*, or per volume or weight of marijuana product.

On May 1, 2013, the definition of THC concentration was changed to state "THC concentration" means percent of delta-9-tetrahydrocannabinol content per dry weight of any part of the plant *Cannabis*, or per volume or weight of marijuana product, or the combined percent of delta-9 tetrahydrocannabinol and tetrahydrocannabinolic acid in any part of the plant *Cannabis* regardless of moisture content.

On June 12, 2014, the definition of “marijuana concentrates” was added to state products consisting wholly or in part of the resin extracted from any part of the plant Cannabis and having a THC concentration greater than sixty percent. The definition of “marijuana-infused products” was changed to state products that contain marijuana or marijuana extracts, are intended for human use, and have a THC concentration greater than 0.3 percent and no greater than sixty percent. The term “marijuana-infused products” does not include either usable marijuana or marijuana concentrates.

On July 24, 2015, the definitions (RCW 69.50.101) of marijuana-infused products and marijuana concentrates changed.

> "Marijuana concentrates" means products consisting wholly or in part of the resin extracted from any part of the plant Cannabis and having a THC concentration greater than ten percent.
>
> "Marijuana-infused products" means products that contain marijuana or marijuana extracts, are intended for human use, are derived from marijuana as defined in subsection (t) of this section, and have a THC concentration no greater than ten percent. The term "marijuana-infused products" does not include either useable marijuana or marijuana concentrates.

Plants of the genus *Cannabis* can be thought of as two distinct varieties – those grown for their fiber content and those grown for their physiological properties. Plants grown for their fiber content are generally referred to as hemp while those grown for their physiological properties are referred to as marijuana in North America or herbal *Cannabis* in Europe. Morphologically these plants are virtually indistinguishable from one another, though the chemical composition of the plants allow for their distinction. Delta-9-tetrahydrocannabinolic acid (THCA) is the biosynthetic precursor to delta-9-tetrahydrocannabinol (THC). THC levels in fresh plant material, regardless of the variety, are variable and the conversion of THCA to THC occurs during drying and with exposure to light and heat. To account for this conversion, the hemp industry has established legal acceptable levels for total THC that reflect the combination of THCA and THC (0.2 percent in Europe; 0.3 percent in Canada). To prevent the probability of false negatives, the identification of marijuana is based on the determination of Total THC and not delta-9-THC concentration alone.

Analysis by Gas Chromatography will result in the conversion of THCA to THC which represents “total THC” in the sample. Although the level of THC is measured, the quantitative examination only serves to help determine if the plant material is marijuana or if the material is a marijuana concentrate.

Any quantitative value of total THC concentration is not representative of the entire exhibit. A sample of the material as received will be selected for analysis. The minimum amount of sample required for determination of THC concentration in leaf marijuana or marijuana concentrates will be 100 milligrams. Exhibits containing less than 100 milligrams of sample will be analyzed to confirm THC and/or THCA.

The Crime Laboratory Division does not provide THC quantitation analysis for marijuana-infused products. However, effective July 24, 2015, infused product cases related to suspects under the age of twenty-one do not require THC quantitation, so we can accept these cases and perform a qualitative analysis.

For all other suspected marijuana-infused product cases in which THC quantitation would be warranted, the customer can be contacted and the case returned or refused. We should then refer them to outside labs approved by the Liquor and Cannabis Board (LCB).

Identification of leaf marijuana and marijuana concentrates in state cases for suspects over the age of twenty-one, and for suspects under 21 until July 23, 2015, will consist of qualitative and quantitative analysis. For cases with an offense date of July 24, 2015 and after, identification of marijuana products for suspects under the age of twenty-one will consist of qualitative analysis.

Measurements of the height and diameter of the plant to establish if a plant or clone is immature will not be conducted by the Crime Laboratory. These measurements need to be taken in the field before collecting and/or packaging of the sample.

25.9.2 ADVANTAGES AND LIMITATIONS

One of the advantages of analyzing marijuana products by gas-chromatography-mass spectrometry is that it minimizes the likelihood of overlooking other substances present in the sample.

Neither GC/MS nor GC/FID addresses the inherent inhomogeneity of the plant.

Gas chromatography does not distinguish THCA from THC unless the sample is appropriately derivatized.

25.9.3 APPARATUS AND EQUIPMENT

- A stereomicroscope with 40x magnification with both reflected and transmitted light is preferable to observe the microscopic characteristics of the plant material.
- GC/MS
- GC/FID
- HPLC
- The Duquenois reagent, hydrochloric acid, and chloroform are necessary for the Duquenois-Levine chemical test.
- THC, Certified Reference Material (CRM)
- THCA, CRM
- Cannabinoid Mix, CRM
- Tribenzylamine (Internal Standard)
- Methanol
- Calibrated pipettes and/or bottle top dispensers
- Calibrated class A Volumetric flasks

25.9.4 PROCEDURE

Case Approach Summary

Qualitative analysis only

- Non-state cases (including Federal and Tribal cases) will be analyzed qualitatively, regardless of the form of the marijuana, the amount of material, the age of the suspect, or the date of the offense.
- Cases with a suspect under the age of 21 and with an offense date on or after July 24, 2015, regardless of the form of marijuana or amount of material.
- Residue cases, regardless of date.
- Cases in which THC was not expected (e.g., exhibits submitted as suspected heroin) that would normally be analyzed quantitatively.
- Leaf and concentrate cases that would normally be quantitatively analyzed but have less than 100 milligrams of material.
- Concentrate and infused product cases with an offense date prior to June 12, 2014.

Qualitative and quantitative analysis

- Leaf marijuana cases that are:
    - More than 100 milligrams;

  - State of Washington cases;
  - Suspect is over twenty-one;
- Marijuana concentrate cases that are:
  - Suspect is under twenty-one and the offense date is July 23, 2015, or earlier.
- Marijuana concentrate cases that are:
  - More than 100 milligrams;
  - State of Washington cases;
  - Suspect is over twenty-one;
  - Suspect is under twenty-one and the offense date is July 23,2015, or earlier.

Analysis – Qualitative

Marijuana Residues
Two techniques, as described in the Seized Drugs Procedure section, will be used to examine smoking devices and residues containing cannabis extracts in order to identify the presence of delta-9-tetrahydrocannabinol or other cannabinoids. Distinguishing delta-9-tetrahydrocannabinol from delta-9-tetrahydrocannabinolic acid is not necessary provided the report clearly states the residue was found to contain THC and/or THCA.

Leaf Marijuana
- Obtain a net weight of the exhibit.
- Microscopic Exam: The plant material is observed under the microscope, looking for the appropriate plant characteristics. The analyst must document their findings in the case notes. Other observations made during the microscopic exam may also be documented.
- Duquenois-Levine Chemical Test (optional):
  - The Duquenois reagent and the material are combined and the mixture is allowed to stand.
  - A few drops of hydrochloric acid are added to the mixture. The reaction is allowed to proceed for no more than two minutes and the color of the solution is recorded. Once the color has formed, a few drops of chloroform are added and mixed. The color of the lower chloroform layer is recorded. A blue to purple color is indicative of cannabinoids.
  - A procedural blank test must be run with each Duquenois-Levine test.
- Gas Chromatography-Mass Spectrometry (optional for non-state cases if Duquenois-Levine Chemical test is performed, required for state cases): A selection of the leaf material will be extracted with appropriate solvent for instrumental analysis.
- A second test to confirm the THC and/or THCA will be performed for exhibits in which THC and/or THCA will be reported.

Marijuana Concentrates
- Obtain a net weight of the exhibit, if possible. Freezing or chilling the sample briefly may assist in removing the material from the packaging. If it is not possible to obtain a net weight the gross weight will be documented and the net weight may be estimated.
- Gas Chromatography-Mass Spectrometry: A selection of the material will be extracted with appropriate solvent for instrumental analysis.
- A second test to confirm the THC and/or THCA will be performed for exhibits containing less than 100 milligrams.

Marijuana Infused Products (Suspects Under Twenty-One)
- Obtain a net weight of the exhibit.
- Extract the material using QuEChERS or other appropriate extraction technique. A preparation blank will also be prepared using the sample technique.

- Two techniques, as described in the Seized Drugs Procedures section, will be used to examine the infused product extracts in order to identify the presence of delta-9-tetrahydrocannabinol or other cannabinoids.

Analysis –Quantitative of Leaf Marijuana and Concentrates

Internal Standard Solution (ISS) Preparation

- Tribenzylamine will be verified by FTIR or GC/MS and the verification data will be maintained.
- Place 2.5 grams of tribenzylamine into a 500 mL Class A volumetric flask and fill to volume with room temperature methanol. Larger or smaller volumes can be prepared as long as the final concentration is 5.0 mg/mL. Record the exact concentration of the Internal Standard Solution.
- Assign a lab lot number to the Internal Standard Solution and store in an amber glass bottle.
- The presence of tribenzylamine in the Internal Standard Solution must be verified by GC/MS. Verification data will be maintained.

Calibration Level Standard Solution Preparation

- Combine ten 1 mg/mL ampoules of certified THC in methanol in a labeled vial or other appropriate container.
- Label six glass tubes or volumetric flasks with the following Calibration Level and lot number:
    - Level 0 – 0 µg/ml THC in methanol
    - Level 1 – 25 µg/ml THC in methanol (2.5x 10^{-4} g/10 mL)
    - Level 2 – 50 µg/ml THC in methanol (5.0x 10^{-4} g/10 mL)
    - Level 3 – 100 µg/ml THC in methanol (1.0x 10^{-3} g/10 mL)
    - Level 4 – 250 µg/ml THC in methanol (2.5x 10^{-3} g/10 mL)
    - Level 5 – 500 µg/ml THC in methanol (5.0x 10^{-3} g/10 mL)
- Add 250 µL of the internal standard solution to each labeled glass tube or flask.
- Add the following amounts of 1 mg/mL certified THC solution in methanol to the labeled Calibration Level glass tubes or flask:
    - Level 0 – no THC solution
    - Level 1 – 250 µL
    - Level 2 – 500 µL (250 µL x 2)
    - Level 3 – 1 mL (1000 µL)
    - Level 4 – 2.5 mL (250 µL x 2 + 1000 µL x 2)
    - Level 5 – 5 mL (1000 µL x 5)
- Add room temperature methanol to bring the volume to approximately 10 mL.
- Add approximately 0.5 mL of each Standard Solution to separate labeled autosampler vials.
- The remaining Standard Solutions will be transferred to labeled glass vials with non-reactive, tightly sealing lids or flame sealed glass ampoules for long term storage in the freezer. The vials or the container holding the vials will be labeled with the expiration date.
- The remaining (approximately 750 µL) of certified THC solution will be added to an autosampler vial and verified by GC/MS. Verification data will be maintained.

Calibration Verification Solution (CVS)

- The Calibration Verification Solution (which will be used to for the Initial Calibration Verification Solution (ICV) and the Continuing Calibration Verification Solution (CCV)) will be prepared from a 1 mg/mL certified reference material THC solution which may be obtained from a separate vendor or the same vendor but from a different lot number than was used for the Standard Solutions. This will be prepared fresh for each analytical batch.

- Add 1000 µL of the certified 1 mg/mL THC solution to a 10 mL labeled glass tube or volumetric flask. Add 250 µL of the Internal Standard Solution and fill to volume with methanol. This will give an approximately 100 ppm solution of THC.
- A CVS dilution up to 4x will be prepared with preparation blank and run in a sequence that contains diluted samples. The remaining THC CRM will be verified by GC/MS and the verification data will be maintained as part of the case record.

Resolution Verification Solution (RVS)

- The Resolution Verification Solution will be prepared from a 1 mg/mL certified reference material mix consisting of cannabidiol (CBD), THC, and cannabinol (CBN).
- Add 1000 µL of the certified 1 mg/mL Cannabinoid mix to a 10 mL glass tube or volumetric flask. Add 250 µL of the Internal Standard Solution and fill to volume with methanol. This will give an approximately 100 ppm solution of CBD, THC & CBN.
- The remaining RVS will be transferred to a labeled glass vial with non-reactive, tightly sealing lid or a flame sealed glass ampoule for long term storage in the freezer. The vials or the container holding the vials will be labeled with the expiration date.
- The remaining CRM will be verified by GC/MS and the verification data will be maintained as part of the case record.

THCA Conversion Solution (TCS)

- The THCA Conversion Solution will be used for sequences containing marijuana concentrates.
- Add 1000 µL of the certified 1 mg/mL THCA solution to a 10 mL labeled glass tube or volumetric flask. Add 250 µL of the Internal Standard Solution and fill to volume with methanol. This will give an approximately 100 ppm solution of THCA.
- The sample amount will be entered into the sequence table as 0.000877 to account for the molecular weight difference of THC and THCA.
- The TCS or remaining THCA CRM will be verified and the verification data will be maintained as part of the case record. THCA will be verified by one of the following analytical schemes:
    - GC/MS with derivatization
    - GC/MS combined with HPLC
    - GC/MS combined with TLC

Case Sample Preparation

- A minimum of 25 mg of leaf material or concentrate will be selected for analysis, weighed, and placed in a glass tube (or vial). A larger sample size may be used if an exhibit is suspected to have a low level of total THC (e.g., an immature plant) or if a dilution is planned. Record the mass of the selected leaf material in the THC Quant Worksheet (CHE-LMW-5031).
- Add 250 µL of the room temperature ISS to the glass tube.
- Add room temperature methanol to bring the volume to approximately 10 mL.
- A preparation blank consisting of 250 µL of the ISS diluted to 10 mL with methanol in a separate glass tube will also be prepared per batch of case samples.
- Sonicate the sample and preparation blank for 15 minutes.
- Allow the sample to settle and filter, if necessary.
- Add approximately 0.5 mL of the sample and the preparation blank to separate labeled autosampler vials.
- Leaf marijuana extracts may be volumetrically diluted with preparation blank prior to analysis. A 2x dilution is recommended.

- Marijuana concentrate extracts will be volumetrically diluted with preparation blank for analysis. A 10x dilution is recommended.

GC/FID Quantitation Procedure

- Samples will be run on the GC/FID utilizing the appropriate quantitation method.
- Prepare a sequence file. For the Calibration levels enter the Sample Type as "Calibration", the appropriate Calibration Level, and "Replace" for Update RF. The Sample Amount of the CVS and RVS should be entered as "1.0e-3" and the Sample Type should be "Sample". The Sample Amount of the TCS (for sequences containing concentrates) should be entered as "8.77e-4" and the Sample Type should be "Sample".
  - System blank
  - Level 1
  - Level 0
  - Level 2
  - Level 0
  - Level 3
  - Level 0
  - Level 4
  - Level 0
  - Level 5
  - Level 0
  - RVS
  - Preparation blank
  - TCS x3 (for batches containing marijuana concentrates)
  - Preparation blank (if dilutions were performed on samples in the sequence)
  - Diluted CVS x3 (if dilutions were performed on samples in the sequence)
  - Initial Calibration Blank (ICB)
  - ICV x3
  - Preparation Blank up to 3x
  - Sample x3

    Sample set
  - Repeat the Sample Set for as many exhibits/items as needed. The Continuing Calibration Blank (CCB) and CCV will be run in triplicate after five Sample Sets and after the final sample in the sequence.
  - Enter the amount in grams of the leaf sample analyzed in the sequence table.

25.9.5 INTERPRETATION AND REPORTING

Qualitative Analysis

Interpretation

A positive microscopic exam for marijuana occurs when cystolithic and simple hairs are observed on opposite sides of the same leaf or leaf fragment.

The Duquenois-Levine test is considered positive for the presence of cannabinoids when a purple to blue color forms after the addition of the hydrochloric acid, followed by a transfer of this color to the organic layer upon addition of chloroform.

Interpretation of data from GC/MS or other analytical techniques will be performed according to the relevant chapter in the technical procedures.

Reporting

Refer to the measurement uncertainty chapter for reporting criteria for sample weights.

Marijuana residue cases, marijuana cases with a suspect under twenty-one (on or after July 24, 2015), and marijuana concentrates and infused products with offense dates prior to June 12, 2014, in which cannabinoids are identified. The report will indicate if THC and THCA are not specifically differentiated.

Example: The analyzed material was found to contain delta-9-tetrahydrocannabinol, tetrahydrocannabinolic acid, and cannabidiol.

Example: The analyzed material was found to contain delta-9-tetrahydrocannabinol and/or delta-9-tetrahydrocannabinolic acid.

Non-state leaf marijuana cases may be reported as found to contain marijuana as defined by the Title 21 United States Code (USC) Controlled Substances Act [21USC802(16)] or in the same manner as state cases in which THC is not quantified.

Example: The analyzed material was found to contain marijuana as defined by the Title 21 United States Code (USC) Controlled Substances Act [21USC802(16)].

If an *analyst not approved to work a THC quantitation case* works a case in which THC is identified and in which THC quantitation may be necessary, the report will state that delta-9-tetrahydrocannabiol (THC) was identified and that analysis to quantify THC, if appropriate for the sample, may be requested by contacting the Crime Laboratory.

Quantitative Analysis

Interpretation

Evaluate the calibration table.

- The correlation must be greater than or equal to 0.9995.

Level 0 and preparation blanks will be evaluated for carryover of THC.

- If the “blank” in the Level 0 or preparation blank immediately preceding the sample contains greater that 1% the peak area (corresponding to THC) of the following sample/standard solution, the Level 0/preparation blank and sample/standard series will be considered invalid and will need to be rerun.

Open the RVS data file. Evaluate the RVS to ensure there is resolution of the three major cannabinoids and the internal standard.

- If there is not resolution the sequence is deemed unacceptable.

Open the ICV data files and evaluate using the ISTD% option. Repeat for each CCV data file in the sequence.

- The average concentration of the CVS must be within 10% of the labeled concentration.
- From the three injections for the sample, calculate the average (AVG), standard deviation (s), and coefficient of variance (CV).
- CV = s/AVG *100
- If the CV is less than 5, the average %Total THC will be accepted for the determination of marijuana. If the CV is greater than 5, replace liner and septum and reanalyze the extract. If the CV is still greater than 5, the extract must be prepared again and analyzed.

Open the TCS data files and evaluate using the ISTD% option. Repeat for each TCS data file in the sequence.

- The average recovery of the TCS injections must be greater than 75%.
- From the three injections for the sample, calculate the average (AVG), standard deviation (s), and coefficient of variance (CV).
- CV = s/AVG *100

- If the CV is less than 5, the average %Total THC will be accepted. If the CV is greater than 5, replace liner and septum and reanalyze the extract. If the CV is still greater than 5, the extract must be prepared again and analyzed.

Open the sample data file and print the report using the ISTD% option. It may be necessary to adjust the threshold or manually integrate low abundant peaks. Enter the results from each sample in the THC Quant Worksheet.

- If the results are less than the low standard, resample and prepare as before but use an appropriately increased amount of leaf material. Enter the amount of leaf sample used in the sequence table. If the results from the second sampling are below the low standard, calculate the Limit of Quantitation of the sampled material and determine if this is below 0.3%. If the Limit of Quantitation is below the 0.3% the data is considered acceptable.
    - Example: 103 mg of material were extracted and the sample was still below the low standard (2.5 x 10^{-4} g/10 mL).

      [0.00025 g/10mL]/[0.103 g/10 mL] *100 = 0.24%
- If the average of the three injections is greater than the high standard, resample and prepare the sample and preparation blank volumetrically, if not originally prepared volumetrically. A diluted CVS must also be added to the sequence. For example, using the sample solution prepare a 2x dilution by combining one part sample with one part preparation blank and include the dilution factor “2” in the sequence table. If other dilution volumes are used, enter the appropriate dilution factor in the sequence table.
- From the three injections for the sample, calculate the average (AVG), standard deviation (s), and coefficient of variance (CV).
- CV = s/AVG *100
- If the CV is less than 5, the average %Total THC will be accepted. If the CV is greater than 5 replace the liner and septum and reanalyze the extract. If the CV is still greater than 5, the extract must be prepared again and analyzed.

If the above criteria are met, record the results for the sample on the THC Quant Worksheet and the calibration, conversion and verification solution results on the Calibration Curve Worksheet.

If any of the above criteria are not met, take appropriate steps (e.g., perform appropriate corrective instrument maintenance, reevaluate the linearity of the instrument, remake the Calibration Level Standard Solutions and/or the Calibration Verification Solution and repeat) to resolve the problem.

Reporting

Refer to the measurement uncertainty chapter for reporting criteria for sample weights.

State leaf marijuana or marijuana concentrates cases requiring THC quantitation:

Approved language for the Remarks section: Marijuana contains both delta-9-tetrahydrocannabinol (THC) and delta-9-tetrahydrocannabinolic acid (THCA). THCA is known to convert to THC upon storage and heating, which occurs during smoking and when analyzed using a gas chromatograph. The identification of marijuana and marijuana concentrates is based on the combined concentrations of THC and THCA (also known as Total THC).

If the *case contains less than 100 milligrams of material* the report will state there was an insufficient amount of material for quantitative analysis.

Example: A sealed plastic bag (item 1) was received. It contained a ziplock plastic bag holding less than 100 milligrams of green plant material. The material was analyzed and found to contain delta-9-tetrahydrocannabinol and/or delta-9-tetrahydrocannabinolic acid.

There was insufficient material for quantitative analysis to determine if the material is marijuana.
Example: A sealed plastic bag (item 1) was received. It contained a ziplock plastic bag holding less than 100 milligrams of green sticky material. The material was analyzed and found to contain delta-9-tetrahydrocannabinol and/or delta-9-tetrahydrocannabinolic acid. There was insufficient material for quantitative analysis to determine if the material is a marijuana concentrate.

Plant material samples:
If the selected sample analyzed is found to contain **0.4% or greater Total THC** and has a positive stereomicroscopic examination, the results will be reported as "marijuana".
Approved language: The material was analyzed and found to contain marijuana based on a Total THC concentration greater than 0.3 percent.

If the sample is found to contain **less than 0.2% Total THC**, the results will be reported as containing "less than 0.3% Total THC".
Example: The analyzed material contains less than 0.3% Total THC.

If the sample is found to contain **more than 0.2% Total THC but less than 0.4% Total THC**, then the report will state the analysis was inconclusive and will include the determined Total THC percentage along with the associated measurement uncertainty. The uncertainty statement will include the coverage factor and coverage probability.
Example: A selection of plant material was analyzed and was found to contain THC, but was inconclusive for marijuana. The material was found to contain 0.26% +/-0.04% Total THC (0.22%-0.30% Total THC). The reported uncertainty is expanded using a coverage factor k=2 for a level of confidence of approximately 95% considering a normal distribution. Contact the Crime Laboratory for additional information.

Example of a state Leaf Marijuana Report:

Results and Conclusions:
The material was analyzed and found to contain marijuana based on a Total THC concentration greater than 0.3 percent.

Evidence:
A sealed plastic bag (item 1) was received. It contained a ziplock plastic bag holding 3.45 grams of green plant material.

Methods and Observations:
The plant material was examined using a stereomicroscope to observe specific physical characteristics. A portion of the material was extracted with methanol and was analyzed by a gas chromatograph/mass spectrometer. A second portion was extracted with methanol containing an internal standard and was analyzed using a gas chromatograph equipped with a flame ionization detector.

Remarks:
Marijuana contains both delta-9-tetrahydrocannabinol (THC) and delta-9-tetrahydrocannabinolic acid (THCA). THCA is known to convert to THC upon storage and heating, which occurs during smoking and when analyzed using a gas chromatograph. The identification of marijuana and marijuana concentrates is based on the combined concentrations of THC and THCA (also known as Total THC).

Concentrate samples:

Cases with offense dates July 23, 2015 or earlier:

The determined Total THC percentage along with the associated measurement uncertainty will be reported for all concentrate exhibits. The uncertainty statement will include the coverage factor and coverage probability.

> Example: A selection of the material was analyzed and was found to contain 63% +/-9.5% Total THC (53.5%-72.5% Total THC). The reported uncertainty is expanded using a coverage factor k=2 for a level of confidence of approximately 95% considering a normal distribution.

Cases with suspects twenty-one or older and offense dates July 24, 2015 or later,
The results will be reported as a marijuana concentrate if the **concentration of THC is greater than 12%.**

> Example: The material was analyzed and found to contain a marijuana concentrate based on a Total THC concentration greater than 10 percent.

If the sample is found to contain **less than 8% Total THC**, the results will be reported as containing “less than 10% Total THC”.

> Example: The analyzed material contains less than 10% Total THC.

If the sample is found to contain **more than 8% Total THC but less than 12% Total THC**, then the report will state the analysis was inconclusive and will include the determined Total THC percentage along with the associated measurement uncertainty. The uncertainty statement will include the coverage factor and coverage probability.

> Example: A selection of the material was analyzed and was found to contain THC, but was inconclusive for a marijuana concentrate. The material was found to contain 9.5% +/-1.4% Total THC (8.1%-10.9% Total THC). The reported uncertainty is expanded using a coverage factor k=2 for a level of confidence of approximately 95% considering a normal distribution. Contact the Crime Laboratory for additional information.

<u>Example of a state Marijuana Concentrate Report for cases prior to July 24, 2015</u>:

> **Results and Conclusions:**
> The analyzed material was found to contain 63% +/-9.5% Total THC (53.5%-72.5% Total THC). The reported uncertainty is expanded using a coverage factor k=2 for a level of confidence of approximately 95% considering a normal distribution.
>
> **Evidence:**
> A sealed plastic bag (item 1) was received. It contained a ziplock plastic bag holding 1.2 grams of brown sticky material.
>
> **Methods and Observations:**
> A portion of the material was extracted with methanol and was analyzed by a gas chromatograph/mass spectrometer. A second portion was extracted with methanol containing an internal standard and was analyzed using a gas chromatograph equipped with a flame ionization detector.
>
> **Remarks:**
> Marijuana contains both delta-9-tetrahydrocannabinol (THC) and delta-9-tetrahydrocannabinolic acid (THCA). THCA is known to convert to THC upon storage and heating, which occurs during smoking and when analyzed using a gas chromatograph. The identification of marijuana and marijuana concentrates is based on the combined concentrations of THC and THCA (also known as Total THC).

Example of a state Marijuana Concentrate Report for adult possession cases after July 24, 2015:

Results and Conclusions:
The analyzed material was found to contain a marijuana concentrate based on a Total THC concentration greater than 10 percent.

Evidence:
A sealed plastic bag (item 1) was received. It contained a ziplock plastic bag holding 1.2 grams of brown sticky material.

Methods and Observations:
A portion of the material was extracted with methanol and was analyzed by a gas chromatograph/mass spectrometer. A second portion was extracted with methanol containing an internal standard and was analyzed using a gas chromatograph equipped with a flame ionization detector.

Remarks:
Marijuana contains both delta-9-tetrahydrocannabinol (THC) and delta-9-tetrahydrocannabinolic acid (THCA). THCA is known to convert to THC upon storage and heating, which occurs during smoking and when analyzed using a gas chromatograph. The identification of marijuana and marijuana concentrates is based on the combined concentrations of THC and THCA (also known as Total THC).

25.9.6 QUALITY ASSURANCE

Newly prepared Duquenois reagent will be reliability checked using positive and negative controls and will be documented in a reagent logbook. Log books shall be maintained identifying the preparer of the reagent and the results of the reliability check of the reagent. The log book entry describing reference materials used to test the reagents must include their source and lot number (if available). One time use reagents need only be documented in the case notes. Monthly checks must be performed using reference materials. Gaps between checks are not a concern during periods of inactivity so long as the performance is checked prior to subsequent use. These checks will be documented in a reagent logbook or case notes.

The GC-FID Quant Worksheet (CHE-FIDQW-5031) will be used to document the analysis of all samples for quantitation cases.

The Calibration Curve Worksheet (MAT-CCW-5034) will be used to document the calibration, conversion and verification solution data.

The Marijuana Qualitative Analysis Worksheet (CHE-MQAW-5035) should be used to document the analysis of all samples for qualitative cases. If this worksheet is not used, measurement uncertainty reporting requirements must be specifically documented in the notes. For example, the weight of the analyzed material was determined to be 24.6 grams. The notes would need to include a statement that MU is NOT required for this item.

The low standard will define the method’s lower limit of quantitation. The high standard will define the upper limit of quantitation.

Weights will be determined using an analytical balance with a readability of 0.0001 gram or better for quantitation cases.

Calibrated pipettes and/or bottle top dispensers will be used for making calibrators, CVS, RVS, TCS, and dispensing ISS during sample preparation.

Volumetric glassware used for the preparation of ISS and calibrators will be calibrated by an external accredited calibration service provider at least every five years.

Prior to using volumetric glassware perform a visual inspection to ensure the glassware is clean and is not chipped or etched. Glassware that is chipped or etched should not be used for the preparation of ISS or calibrators.

All lot numbers and verification data will be maintained for THC quantitation as part of the case record.

The calibrators and RVS will be assigned a lab lot number and an expiration date which is not to exceed the manufacturer's expiration date for the certified reference material.

Frozen/refrigerated solutions or solvents will be allowed to warm to room temperature before use.

The CVS will be used to confirm the calibration curve and will be prepared fresh for each analytical batch.

The RVS will be used to provide retention time data for cannabidiol and cannabinol and to establish resolution of cannabidiol and tribenzylamine.

The TCS will be used to evaluate the conversion of THCA to THC and will be prepared fresh for each analytical batch in which a concentrate case is analyzed.

The Calibration Levels Standard Solutions will be evaluated for conversion of THC to cannabinol.

25.9.7 SAFETY

Hydrochloric acid and chloroform are inhalation hazards and should be used with proper ventilation. In addition, chloroform is a carcinogen and hydrochloric acid is very corrosive. Analysts should be familiar with the information listed in the Safety Data Sheet (SDS) for each chemical utilized in this technique.

25.10 **DETERMINATION OF TOTAL THC CONCENTRATIONS IN LIQUIDS**

25.10.1 INTRODUCTION

This procedure is an extension of the existing marijuana identification procedure designed to address the differences in analyzing a liquid containing an unknown concentration of THC and is intended to be primarily used with liquid proficiency samples that are in methanol or other solvents. The general procedure, data analysis/interpretation, and quality assurance measures will be the same as the marijuana quantitation procedure.

25.10.2 ADVANTAGES AND LIMITATIONS

Liquid samples can be assumed to be homogenous and the results will be representative of the whole sample and not just the portion which was analyzed.

25.10.3 APPARATUS AND EQUIPMENT

As described in the marijuana analysis procedure.

25.10.4 PROCEDURE

Liquid samples should be kept in the freezer when not being directly handled for analysis.

The stock 5.0 mg/mL Internal Standard Solution (ISS) will be used to prepare a 500 µg/mL internal standard solution. Use 100 µL of the 5.0 mg/mL ISS and dilute with 900 µL of MeOH.

Consider the total amount of liquid in the sample and use less than half of the total volume for the analysis. Samples consisting of approximately one milliliter of total volume can likely be analyzed by using 150 µL for the quantitation sample and 100-150 µL for the GC/MS sample. This volume may need to be adjusted due to the nature of the sample. Dilution of the sample for GC/MS is likely not necessary and a conical vial or insert in a standard vial should be used. The following is an example of a quantitation sample preparation:

Preparation blank:
- 100 µL 500 µg/mL ISS
- 300 µL MeOH

Sample:
- 150 µL sample
- 100 µL 500 µg/mL ISS
- 150 µL MeOH

The Calibration Verification Solution (CVS) will be prepared at 375 µg/mL using the 500 µg/mL internal standard solution (ISS). A CVS dilution as described in the marijuana quantitation procedure will be analyzed if diluted samples are to be analyzed. The following is an example of volumes which could be used to prepare the CVS:
- 100 µL 500 µg/mL ISS
- 150 µL 1000 µg/mL THC CRM
- 150 µL MeOH

A THCA Conversion Sample (TCS) will be prepared at 100 µg/mL using the 500 µg/mL internal standard solution (ISS). The sample amount may be left blank in the Sequence Table for the TCS or if a value is entered the molecular weight difference of THC and THCA should be considered. The following is an example of volumes which could be used to prepare the TCS:
- 375 µL 500 µg/mL ISS
- 150 µL 1000 µg/mL THCA CRM
- 975 µL MeOH

The THC quant method on the GC/FID will be adapted as follows and saved as a method with a different name:

- The amount will be changed from g/10mL to µg/mL.
- The report type will be changed from ISTD% to ISTD.
- The calibrator and internal standard values used in the calibration table will be entered as:

	Concentration of THC		**Concentration of Internal Standard**	
Level	**Standard Method**	**Liquid Method**	**Standard Method**	**Liquid Method**
Units	**g/10 mL**	**µg/mL**	**g/10 mL**	**µg/mL**
1	2.5e-4	25	1.25e-3	125
2	5.0e-4	50	1.25e-3	125
3	1.0e-3	100	1.25e-3	125
4	2.5e-3	250	1.25e-3	125
5	5.0e-3	500	1.25e-3	125

25.10.5 INTERPRETATION AND REPORTING

Interpretation

Follow the standard marijuana method for general data analysis and interpretation including using an Excel spreadsheet to calculate the average concentration, standard deviation and coefficient of variance for the triplicate injections of CVS, TCS, and samples. The average amount determined for the analyzed sample will be represented in µg/ml and must be adjusted for dilution of the sample preparation to determine the concentration of the original liquid sample. This calculation can be done in the Excel spreadsheet or manually in the notes page. The following formula will be used to derive the original sample concentration:

$$\text{Original sample conc.} = \frac{\text{average prepared sample conc. * final prepared sample volume}}{\text{volume of original sample aliquot}}$$

Example: A 150 µL aliquot of liquid containing an unknown concentration of THC was used to prepare a 500 µL sample for analysis. Triplicate injections of this prepared solution were averaged and determined to be 225 µg/mL (this value is taken from the data analysis spreadsheet).

$$\text{Original sample conc.} = \frac{225\ \mu g/mL * 500\ \mu L}{150\ \mu L} = 750\ \mu g/mL$$

Measurement uncertainty will be calculated and documented in the case notes.

Reporting
Proficiency tests will be reported in the format stipulated by the Proficiency Test (PT) Provider. If the PT provider has a narrative section in the PT Data Entry Form (or online equivalent), general reporting guidelines from the marijuana chapter will be followed. The narrative style of reporting for liquid PT samples will state the determined Total THC concentration of the sample as well as measurement uncertainty along with the coverage factor and coverage probability.

25.10.6 QUALITY ASSURANCE
As described in the marijuana analysis procedure.

25.10.7 SAFETY
As described in the marijuana analysis procedure.

25.11 **MEASUREMENT UNCERTAINTY**

25.11.1 INTRODUCTION

The International Vocabulary of Basic and General Terms in Metrology (VIM) defines uncertainty as “a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand.”Measurement is an estimation of “true value” due to the sources of variation that influence weighing and purity determination. While it is impossible to account for every variable that could potentially affect the determination of “true value”, an effort will be made to take into consideration the greatest sources of variability and their influence on weighing and purity determination.

25.11.2 DETERMINING UNCERTAINTY OVERVIEW

The eight step process outlined in the Guide to the Expression of Uncertainty of Measurement (GUM) and the National Institute of Standards and Technology (NIST) will be followed to determine an expanded uncertainty. The steps include:

Specify the measurement process

- Identify and characterize uncertainty sources
    - A Cause and Effect Diagram or list can be used to identify the measurement influence factors and to show their relationship to the other factors
    - Each of the measurement influence factors identified will be evaluated to determine their impact on uncertainty
- Quantify uncertainty estimates
    - All units of measurement for the influence factors with significant impact on uncertainty must be made to match using conversion values or coefficient equations.
- Convert factors to standard uncertainties
    - Determine the type of distribution (normal, rectangular, triangular, etc.) applicable for the influence factors.
- Calculate combined standard uncertainty
- Expand the uncertainty by k
    - k=2 will be used for expanded uncertainty
- Evaluate the expanded uncertainty
- Report the uncertainty

The Measurement Uncertainty Estimation Template provided by ASCLD/LAB will be used to document measurement uncertainty calculations. SI units of measurement will be used for determining uncertainty. Documentation of traceability will be maintained when quantifying uncertainty estimates. Traceability as defined by Quantifying Uncertainty in Analytical Measurement (QUAM) is the property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all have stated uncertainties.

25.11.3 DETERMINING UNCERTAINTY FOR BALANCES

- Specify the measurement process
    - The following formula applies to calculating measurement uncertainty in balances:
      y=(mx+b)+U where:
      y is the balance indication;
      m is the sensitivity of the weighing device;
      x is the applied load;
      b is the zero offset, and
      U is the assigned measurement uncertainty (2* u_c).
      (u_c is the combined standard uncertainty)
- Identify and characterize uncertainty sources
    - Uncertainty sources will be listed on “Components & Evaluation” sheet
- Quantify uncertainty estimates
    - When establishing measurement uncertainty, each analyst will weigh two surrogate samples representing approximately 5% of the balance capacity and approximate balance capacity (or appropriate lower capacity) on each balance in the laboratory at a minimum of once a month. If the balance capacity is excessively large, a sample less than balance capacity may be used. Surrogate samples will consist of either a powder or other appropriate material sealed into a bag to prevent sample loss.
    - If a new analyst joins the group they must complete the measurement uncertainty training and will weigh surrogate samples for a minimum of six times before the measurement uncertainty is recalculated. These six weighing events can be weekly or monthly.
    - A minimum of twelve data points per balance will be recorded before the process standard deviation is calculated.

- Surrogate samples will be weighed quarterly by all seized drug analysts and the measurement uncertainty will be updated annually.
- Convert factors to standard uncertainties
- Calculate combined standard uncertainty
  - Use the following formula to calculate combined standard uncertainty;

    $u_c = \sqrt{s_p^2 + r_z^2 + r_{40}^2 + b_c^2 + l_i^2}$

    Where:
    s_p = process standard deviation calculated from surrogates
    r_z = readability at zero
    r_{40} = readability at 40 grams
    b_c = balance calibration uncertainty
    l_i = linearity
- Expand the uncertainty by k
  - k = 2 will be used for expanded uncertainty
  - $U = k*u_c$
- Evaluate the expanded uncertainty
  - In order to evaluate the expanded uncertainty, the following questions should be answered.
    - Does the expanded uncertainty make sense?
    - Does it seem appropriate for the balance?
    - Does it meet the requirements of the laboratory process?
- Report the uncertainty
  - Multiple Weighing events
    - When combining weights to report a total net weight of multiple packages, one cannot simply add together the individual uncertainties and report the combined uncertainty. All calculations that are necessary to calculate combined uncertainty and the total weight of exhibits must be documented in the case notes. If weights are to be combined and one uncertainty reported, the same balance will be used for weighing each package.

      $U = \sqrt{N*(u_b)^2}$ or $\sqrt{N}$ * ub
      U = total Uncertainty
      N = number of measurements
      ub = Uncertainty of the balance
    - Dynamic and static weighing are both considered two weighing events and the reported uncertainty must account for the two weighing events. This calculation may be done manually with the above listed formula or the MU spreadsheet can be updated to automatically perform this calculation. For balances with more than one range, the weighing event related to the tare of the weighing vessel will use the lower weight uncertainty.
    - Examples:
      - A bag of leaf material weighed 39.8 grams which is in the lower range of the balance..

        $U = \sqrt{N*(u_b)^2} = \sqrt{2*0.0062g^2}$ = 0.0088 g
      - A exhibit of baked goods weighed 452 grams which is in the upper range of the balance.

        $U = \sqrt{(u_{bl})^2 + (u_{bu})^2} = \sqrt{(0.0062g)^2 + (0.013g)^2}$ = 0.014 g

        U = total Uncertainty
        u_{bl} = Uncertainty of the balance lower range

u_{bu} = Uncertainty of the balance upper range

- Five plastic bags of marihuana were tested and the total net weight was determined to be 42.5000 grams. The same balance and same range was used for each of the five bags of marihuana.

  U = $\sqrt{N*(u_b)^2}$ = $\sqrt{10*0.0062g^2}$ = 0.020 g

25.11.4 DETERMINING UNCERTAINTY FOR PURITY CALCULATIONS

- Specify the measurement process
  - A generic equation representing the measurement process:
    Y = y + b ± U
    Y is the true value
    y is the measurement result
    $y = f(x_1, x_2, \ldots, x_n)$
    b is bias
    U is expanded uncertainty
- Identify and characterize uncertainty sources
  - Uncertainty sources will be listed on “Components & Evaluation” sheet
- Quantify uncertainty estimates
  - The results from the analysis of the Calibration Verification Solution will be used to establish the Measurement Process Reproducibility (%RSD).
  - Tetrahydrocannabinolic acid (THCA) Certified Reference Material will be evaluated for conversion to tetrahydrocannabinolic acid (THC) and the resulting standard deviation of these values will be used to represent the THCA to THC Conversion.
  - Uncertainty values for the Calibrator will be used from the Certificates of Analysis
  - Pipette and Volumetric flask values will be used from the Certificates of Calibration
  - Balance uncertainty will be laboratory established measurement uncertainty values
- Convert factors to standard uncertainties
- Calculate combined standard uncertainty
  - Use the following formula to calculate combined standard uncertainty:

  $u_c = \sqrt{t_c^2 + t_v^2 + u_p^2 + u_b^2 + u_v^2 + s_p^2 + u_c^2}$

  Where:
  t_c = THC calibrator process standard deviation
  t_v = THC verification solution process standard deviation
  u_p = pipette uncertainty
  u_b = balance uncertainty
  u_v = volumetric flask uncertainty
  s_p = process standard deviation calculated from surrogates
  u_c = THCA to THC conversion process standard deviation

- Expand the uncertainty by k
  - K = 2 will be used for expanded uncertainty
  - $U = k*u_c$
- Evaluate the expanded uncertainty
  - In order to evaluate the expanded uncertainty, the following questions should be answered:
    - Does the expanded uncertainty make sense?

    - Does it seem appropriate for the method?
    - Does it meet the requirements of the laboratory process?
- Report the uncertainty
- Uncertainty will be updated for each batch of calibrators

25.11.5 REPORTING UNCERTAINTY

Marijuana is the only controlled substance in the State of Washington in which the penalty is dictated by the amount of substance in possession. The following weights are significant for recreational marijuana and measurement uncertainty will be reported for exhibits weighing within the indicated ranges:

Marijuana Form	Statutory Value	MU Reporting Range
Leaf	½ ounce (14 grams)	13-15 grams
	1 ounce (28 grams)	27-29 grams
	40 grams	39-41 grams
Infused Product, Solid	8 ounces (227 grams)	224-230 grams
	16 ounces (453 grams)	449-459 grams
Concentrate	3.5 grams	3.3-3.7 grams
	7 grams	6.8-7.2 grams

The case notes must contain the measurement uncertainty or a statement indicating that it was not necessary. The case report will clearly reflect the weight stated with the expanded uncertainty and include the coverage factor and the coverage probability.

Reporting requirements for THC purity are described in the marijuana chapter.

The rounded expanded uncertainty shall be reported to at most two significant digits. Microsoft Excel rounding rules will be followed.

Measurement uncertainty for static weighing processes and the weighing of multiple exhibits of material with a reported combined weight will be calculated using the formula described in the Uncertainty Calculations section of the Seized Drugs Training Manual - Measurement Uncertainty Chapter.

For leaf marijuana or marijuana products outside of the above mentioned ranges or any other seized drug, uncertainty will not be reported unless specifically requested by the customer. If a request for measurement uncertainty is made after the initial report has been issued, a second request will be generated. The notes for the second request will include the original weight and balance used to determine the weight as well as the measurement uncertainty. The case report will clearly reflect the weight stated with the expanded uncertainty calculated using the coverage factor k = 2 and the coverage probability.

Example:
The measured result is 40.5000 grams ± 0.0062 gram. The reported uncertainty is expanded using a coverage factor k=2 for a level of confidence of 95.45%, assuming a normal distribution.

25.12 PHARMACEUTICAL IDENTIFICATION

25.12.1 INTRODUCTION

Commercially manufactured pharmaceutical preparations are typically imprinted or marked by the manufacturer. Pharmaceutical preparations include but are not limited to tablets, capsules, injection vials, patches, suppositories, etc. Manufacturer's markings along with the color and shape of the pharmaceutical preparations can be used as a category 2 test for the analysis of these materials. Labeling from intact factory sealed pharmaceutical packaging may also be used.

25.12.2 ADVANTAGES AND LIMITATIONS

The use of pharmaceutical identification is a rapid, non-destructive test. This technique does not provide molecular structural data and is therefore considered to be a Category 2 test for the identification of seized drugs. This method cannot be used if markings are not present or are indistinct.

25.12.3 APPARATUS AND EQUIPMENT

This technique requires the use of reliable reference material. A microscope may help in the visualization of physical characteristics on the pharmaceutical preparations.

25.12.4 PROCEDURE

Non-factory sealed:
The pharmaceutical preparation is physically described, with the aid of the microscope if necessary. A detailed description of the physical characteristics of the product such as the color, shape, scoring and type of pharmaceutical preparation, as well as the markings on the preparation are recorded.

These characteristics are then compared to a literature reference. Suitable references include, but are not limited to, the Drug Identification Bible, The Physician's Desk Reference, Ident-a-Drug, the DEA Logo Index, the RX-ID CD ROM published by Amera-Chem, Drugs.com, and manufacturers' provided information.

Factory sealed:
Information on the label of factory sealed preparations may also be used as an identifier and will be recorded in the case file. No additional literature reference is required for this preparation.

25.12.5 INTERPRETATION

The color, shape and markings of the pharmaceutical in question must match the description in the literature. Colors are subjective and the analyst may take that into consideration. For instance, the literature may list a tablet as peach, and the analyst may describe the color as orange. There is reasonable agreement between those color descriptions. Information on the scoring of tablets is not always available in the literature references. When available the scoring should be compared, but it is not necessary if the information is not available in the reference material. If the logo, color and shape are associated with more than one pharmaceutical preparation, the reference is not suitable for identification purposes. Literature comparisons will be documented in the case notes and report.

Pharmaceutical identification must be coupled with a category 1 test for a conclusive identification of the substance. If the analyst chooses not to confirm the identity of the substances purported to be in the pharmaceutical preparation from researched logo information, they have the option of reporting only the information generated from their logo research, as long as it is clearly stated that the information has not been analytically confirmed. An example of a qualified report could state: "A sealed plastic bag containing ten white tablets (imprint: SEROQUEL 200) was received. Based on visual characteristics, available references indicate the tablets are consistent with a pharmaceutical preparation containing quetiapine fumarate. The tablets' active ingredient was not analytically confirmed."

A copy of the literature reference must be included with the case file. If using factory sealed package labeling information, a photocopy or photograph of the package will be included in the case file.

Special Cases
When chemical analysis is conducted on intact pharmaceutical preparations with controlled substances occurring in multiple schedules, the analyst must determine the presence of active, non-narcotic ingredients. If present, the active non-narcotic ingredient(s) will be reported. In addition, where pharmaceutical identifiers/markings indicate concentrations of active ingredients, the report will specify these values. An example of a report for Vicodin® tablets: Ten tablets bearing like physical markings which indicate the tablets contain 10 mg hydrocodone and 500 mg acetaminophen. One tablet was analyzed and was found to contain dihydrocodeinone and acetaminophen.

For cases where chemical analysis is not conducted to confirm the active ingredients in such preparations, reporting of the ingredients will be qualified as appropriate for the level of analyses performed. Referring to the above example of Vicodin® tablets: Ten tablets bearing like physical markings which indicate the tablets contain 10 mg hydrocodone and 500 mg acetaminophen. The tablets were not further examined.

Hydrocodone/dihydrocodeinone may be reported as dihydrocodeinone or hydrocodone if the dosage unit is intact and the markings are consistent with RCW 69.50.208 (Schedule III). If the exhibit is in a form other than an intact dosage unit, the analyst may report hydrocodone or dihydrocodeinone or a combination of both.

Propoxyphene will be reported as such, unless a clear determination of chirality has been made. A statement will be made in the report that only dextropropoxyphene is controlled by law and that the precise isomer was not determined in this case. An example of a qualified statement: “The tablet was found to contain propoxyphene. Logo identification indicates the tablet contains dextropropoxyphene, which is the controlled form. No further analysis was performed to determine the form of propoxyphene present.”

Ephedrine and/or pseudoephedrine can be reported as the specific isomer based on a logo identification and a GC/MS that does not specifically resolve the two.

25.12.6 SAFETY
There are no specific safety precautions associated with this technique.

25.13 **QUECHERS**

25.13.1 INTRODUCTION

Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe (**QuEChERS)** are a method of extracting drugs from complex matrices (e.g., candy/chocolate concoctions, baked goods, infant formula). Co-extractive compounds associated with these matrices (e.g., fats, cholesterols, sugars) tend to carry through with the target analytes when common extractions are employed. While originally designed for extraction and cleanup of pesticide residue samples, the technique has been shown to be effective for seized drug analysis.

25.13.2 ADVANTAGES AND LIMITATIONS

This is a quick, cost effective technique for the isolation of drugs in complex matrices. Drugs which are traditionally found in very low concentrations (e.g., psilocyn in chocolates, LSD on candy) may not be extracted effectively using this technique. QuEChERS have been shown to be particularly effective in the isolation of cannabinoids from food products for qualitative analysis.

25.13.3 APPARATUS AND EQUIPMENT

Many companies produce QuEChERS extraction salts and associated equipment. The manufacturer's recommended consumables should be acquired for use with the extraction salts. Homogenization of the sample is required and may be accomplished with equipment other than a dedicated homogenizer. Additionally, a centrifuge, vortex, and lab shaker/rocker may be necessary.

25.13.4 PROCEDURE

The following is a procedure which may be used with the Restek Q-sep™ Q110/Q211 dSPE system. The manufacturer's recommended procedures should be followed if using a QuEChERS system from a different manufacturer.

Sample Extraction

- If the sample is liquid in nature, homogenize until a uniform representative sample is achieved. If the sample is solid in nature, weigh an appropriate amount of sample and dissolve in enough deionized water to an end point of 5 milliliters of homogenized sample. Mechanical homogenization may be necessary.
- A preparatory blank is created using 5 milliliters of deionized water and will be processed concurrently with the sample.
- Place the 5 milliliters of homogenized sample in a 15 milliliter plastic conical tube.
- Add 5 milliliters of acetonitrile to tube.
- Cap the tube and shake by hand for one minute.
- After mixing, vortex the tube.

Sample Drying

- Divide the Q-sep™ Q110 packet into two 3.25 gram portions of powder. (The packet originally contained 6.5 grams of salts).
- Pour one portion of the salts into the blank sample and the other portion into the sample. Shake for at least one minute. Alternatively the tubes can be placed on a lab rocker/shaker for approximately 30 minutes for more consistent mixing.
- Place the tubes into a centrifuge and spin on a high setting for 3-5 minutes.
- Remove the top layer (acetonitrile). If the layer is clear it can be analyzed as is. If there are particulates or a distinct color in the acetonitrile, further cleanup may be necessary.

Optional Drying/Cleanup

- For further drying or sample cleanup, 1 milliliter of the acetonitrile extract may be transferred to ta 2 milliliter Q-sep™ tube. The preparatory blank should be treated in the same manner as the sample.
- After mixing for one minute, place the Q-sep™ tubes in a centrifuge and spin on a high setting for one minute.
- The acetonitrile is ready to be analyzed. Additional filtering may be necessary for analysis by LC.

25.13.5 SAFETY

The addition of QuEChERS salts to the homogenized sample is a rapid exothermic reaction and the conical tubes will heat rapidly. Use of gloves to prevent burns may be necessary.

Aqueous homogenized samples should be near neutral upon addition of the QuEChERS salts. A rapid evolution of gas occurs when the QuEChERS salts interact with bicarbonate bases within the conical tube.

26 CLANDESTINE LABORATORY ANALYSIS

26.1 INTRODUCTION

The purpose of these procedures is to describe how evidence from suspected clandestine laboratories is approached analytically in the Washington State Patrol Crime Laboratory. The objective in analyzing such evidence is to determine if drugs of abuse had been, are being, or could be manufactured, the synthetic route utilized, and the production capacity.

The most common drug of abuse manufactured in clandestine laboratories in Washington is methamphetamine. However, clandestine laboratories involved in the production of other drugs of abuse including, but not limited to, 3,4 - methylenedioxymethamphetamine (MDMA), methcathinone, lysergic acid diethylamide (LSD), phencyclidine (PCP), phenethylamines, tryptamines, and other controlled substance analogs, may be encountered. Evidence from a clandestine drug laboratory may include seized drugs, precursors, chemical reagents, solvents, by-products, and chemical waste. Due to the diversity of evidence encountered, individuals should be fully trained in the analysis of seized drugs before analyzing evidence in this discipline.

26.1.1 ANALYTICAL TECHNIQUES

The analysis of clandestine laboratory evidence will draw upon the knowledge and training of a forensic chemist in analysis of both organic and inorganic materials. Due to the wide variety of samples submitted in clandestine lab evidence, an analyst's analytical approach routinely varies from sample to sample. Observation of physical characteristics such as, but not limited to: state of matter, color, particle size, density, and hetero/homogeneity will often help determine the analytical approach one takes to characterize a substance. Chemical properties such as pH, miscibility, and solubility, will also guide an analyst's approach to a sample.

The analysis of clandestine laboratory evidence utilizes a variety of instrumentation, including but not limited to, GC, GC/MS, FTIR, RAMAN, XRF, SEM/EDX, and CE. An analyst must be trained and approved in the use of these instruments prior to using them in casework. Technical procedures for these instruments are documented other sections of this manual.

Additionally, non-instrumental tests such as flame tests, color and precipitation tests, and microcrystal tests will also aid a clandestine lab analyst in the identification of evidence. Further information on these tests and their use in clandestine drug evidence are documented in the Clandestine Laboratory Training Manual Appendices and other sections of this manual.

26.1.2 TERMS AND DEFINITIONS

By-Products
Substances, other than the intended product, that are formed as a result of a chemical reaction. By-products may or may not be manufacturing method specific.

> For example:
> 1-(1,4-cyclohexyldienyl)-2-methylaminopropane (CMP or 150) is a method specific by-product from the liquefied ammonia and alkali metal method of methamphetamine manufacture.
>
> 1,3-dimethyl-2-phenylnapthalene and 1-benzyl-3-phenylnapthalene (232's), are by-products consistent with the phosphorus and iodine method of methamphetamine manufacture.

Essential Chemicals
Essential chemicals are chemical reagents which are combined with precursor material in an effort to synthesize an intended product. An analyst typically uses the identification of essential chemicals to form

an opinion when reporting a specific clandestine method or synthesis route. These materials may be identified in a pre-synthesis form (e.g., solid iodine, lithium metal) or in a post-synthesis form (e.g., iodide in an aqueous solution, lithium salts).

Examples of essential chemicals: red phosphorus, iodine, ammonia, lithium metal, hypophosphorous acid, hydriodic acid.

Instrumental Test
An instrumental test uses a scientific instrument to obtain reviewable information or data. An instrument may or may not provide structural information. Some examples of instrumental tests are FTIR, RAMAN, XRF, SEM/EDX, CE, GC, GC/MS.

Non-Instrumental Test
A non-instrumental test is one that elicits a visible change. Examples of non-instrumental tests include pH, color tests, formation of a precipitate, microcrystal tests, and flame tests. They do not include physical properties such as appearance, odor, solubility, miscibility, or evaporation rates.

"Other Chemical Methods"
A phrase used in reports to describe all non-instrumental and wet chemical tests used during analysis.

Precursor
A substance from which an intended product is formed. A list of controlled substance precursors can be found in the Revised Code of Washington, Chapter 69.43, The Washington Administrative Code Chapter 246-889-020 and the Code of Federal Regulations Section 1308.47. (Examples: pseudoephedrine, ephedrine, phenylpropanolamine).

Product
A substance that is intentionally formed as the result of a reaction involving chemical reagents and one or more precursors. Clandestine lab products are typically controlled substances or controlled substance analogs.

Qualified Result
When used in the context of a clan lab report, phrasing such as "consistent with…", "analysis indicates…", "detected the presence of…" or similar, to convey that the results of the testing process did not conclusively identify a substance.

Supplementary Chemicals
Supplementary chemicals are additional chemicals that may be associated with clandestine lab evidence, but are not considered essential chemicals. These chemicals are generally not method specific, may be attributed to more than one method, or are not associated with clandestine lab evidence. For analytical purposes, supplementary chemicals include by-products. (Examples: tablet excipients, solvents, sodium hydroxide, hydrochloric acid).

26.2 **ADVANTAGES AND LIMITATIONS**

Due to the variable nature of the evidence received in clandestine laboratory analysis casework, a single approach or set of methods cannot adequately address all possible contingencies. The technical procedures in this document are therefore understood to be flexible to the requirements of each particular case.

26.3 **APPARATUS AND EQUIPMENT**

Clandestine laboratory analysis utilizes a combination of analytical techniques. Part Two of this manual discusses in detail the Instrumentation and Techniques which are available to the scientist.

26.4 PROCEDURE

26.4.1 DETERMINATION OF WEIGHTS AND VOLUMES

All evidence items and samples that are analyzed will be weighed or measured as appropriate in accordance with the Chemical Analysis Technical procedures. Often clandestine laboratory evidence includes items that are sampled in duplicate from the original, and divided between two or more air-tight cans (e.g., can 'A' and can 'B'). Weight or volume measurements of these samples may not accurately reflect that of the original evidence item. It is therefore acceptable to estimate the volume or weight of these samples at the discretion of the scientist. This estimate may be in typical measurement units such as grams or milliliters, or may be based on a description such as: One glass vial 2/3 full of brown liquid. It should also be noted that many items and samples in a clan lab can be hazardous to the balance, and therefore weight or volume should be estimated if this is suspected.

26.4.2 ANALYSIS

Clandestine lab case samples may contain a variety of liquids, solids, pure reagents, reaction mixtures, extracts, by-products, and waste chemicals. Samples will normally be collected at the scene in duplicate to ensure that sufficient samples are available for reanalysis if required; therefore, only one sample set needs to be examined. When the analyst has knowledge that only one sample was collected, half or more of the sample must be preserved for future analysis or appropriate written approval must be obtained per the Crime Laboratory Division QOM, Limited Sample section.

It may not be necessary to analyze every item of evidence submitted in order to determine whether the clandestine manufacture of a drug of abuse had occurred or which method was used. It is the responsibility of the analyst to perform sufficient testing to support any reported conclusions, including performing appropriate blanks and controls. No statistical sampling plan is necessary as quantitative analysis will not be performed.

The identification of a chemical is made when a scientist has acquired enough information about the chemical to distinguish it from all other chemicals. In general, a successful chemical identification strategy will utilize two or more techniques which lead to the same conclusion and preclude a false positive identification. The analyst must follow the analytical procedures outlined in the Clandestine Laboratory Training Manual, the Materials Analysis Technical Procedures, the Seized Drug Training Manual, a published reference, or a new method that follows the validation procedures of the QOM, Technical Procedures and Methods. The use of a procedure from a published reference must be verified prior to use in casework.

Additional information may be gained through additional types of testing in which the Clandestine Laboratory analyst may not have proficiency. If an item requires analysis that falls outside the analyst's current field of expertise, the item should be submitted to a scientist experienced in the requisite discipline. An analyst proficient in the relevant area may be consulted regarding safety concerns, appropriate packaging of the material, screening tests, or other information. After technical review by another analyst proficient in the technique, the Clandestine Laboratory analyst may use this identification in the course of the report as part of the narrative describing the manufacturing process.

Seized Drugs and Controlled Substance Precursors:
The identification requirements of seized drugs are addressed in the Seized Drugs chapter and applies to chemicals listed in the Federal and/or Washington State Uniform Controlled Substances Acts. These requirements will also pertain to substances determined to be controlled substance precursors as defined by the Revised Code of Washington.

Non-Drug Substances:
Non-drug substances in clandestine laboratories include solvents, acids, bases, chemical reagents, inorganic materials, by-products, and chemicals not related to the manufacture of a drug of abuse. These substances, while not specifically scheduled, often provide critical information in the analysis of clandestine laboratory evidence and their identification may be necessary to form a more definite

conclusion. For analytical purposes, identification of non-drug substances in clandestine laboratory casework has been divided into two categories: essential chemicals and supplementary chemicals.

Essential Chemicals:
To report an essential chemical, an analyst must perform a minimum of two tests supporting his/her conclusion, one of which must be instrumental. If none of the testing is instrumental or if the testing conducted does not sufficiently identify the substance, the result may be reported, but the result must be qualified in the report.

Instrumental tests that do not give a positive result, but narrow the possibilities of a substance's identity will be considered equivalent to a non-instrumental test in instances where additional testing provides a positive test. For example, an item would be "consistent with lithium metal" using XRF (considered non-instrumental for lithium) and observing a positive flame test for lithium. Methodologies for analyzing essential chemicals are found in the appendices of the Clandestine Laboratory Training Manual.

Supplementary Chemicals:
To report a supplementary chemical, an analyst must perform either one instrumental test that provides structural information or perform a minimum of two tests supporting his/her conclusion, one of which must be instrumental. If none of the testing is instrumental or if the testing conducted does not sufficiently identify the substance, the result may be reported, but the result must be qualified in the report. Refer to Table 1: Categories of Analytical Techniques in the Seized Drugs chapter for a list of instrumentation that provides structural data.

Other Materials:
Materials that an analyst determines not to be related to drugs of abuse manufacture do not require a complete analysis. The report should reflect the limited analysis (using appropriate language such as 'The material was screened…') and any appropriate conclusions should be made using qualifying language.

Incomplete Identification:
It may not be possible, or even necessary, for every chemical of interest to be conclusively identified in every case. When the data are sparse, very limited in scope or cannot serve as the basis for more definite conclusions, it may not be appropriate to mention the suspected presence of the material in the report. It should, however, be documented in the notes. Chemicals that are not conclusively identified may be considered in evaluating and interpreting the overall circumstances of the case, particularly in regard to how they may be part of a synthesis scheme or how they are related to the other evidence in the case. Their value in this regard, however, should be properly considered since they have not been conclusively identified, and this should be reflected in the conclusions that are drawn

26.4.3 DOCUMENTATION
Case notes and reports must follow the procedures set forth in Section 3 of this manual. Notes must be legible and support the conclusions of the analyst.

Sample identifiers (#1, 4A, B13, etc.) and a physical description of each sample will be included in case notes. Evidence from clandestine laboratories can change over time through evaporation of solvents, by-product formation, or other chemical reactions. Physical descriptions may not necessarily coincide with those recorded during evidence collection. Color photographs of samples may be taken to document the appearance of evidence or for courtroom display. Refer to QOM, Digital Images in Casework Documentation regarding preservation of digital images in casework.

26.4.4 ESTIMATING YIELD CAPACITY
If yield capacity is reported, it will be reported as a theoretical yield and must be properly explained or qualified in the report. All calculations made in determining yield must be included in the notes.

26.5 INTERPRETATION AND REPORTING

26.5.1 INTERPRETATION

Interpretation of analytical data and observations allows an analyst to summarize his/her findings in a written conclusion. An analyst may also draw upon training, experience, discussions with colleagues, and relevant reference material to interpret casework and form a basis for conclusion. The analyst should be aware of the variety of manufacturing methods into which a particular sample may fit. Additionally, not all items may be able to be attributed to the manufacture of a drug of abuse.

The analytical results are individually and collectively evaluated as to their significance in the manufacture of a drug of abuse. Due to the complexity of clandestine lab casework it is important to understand what significance can be applied to each piece of data.

Information regarding other materials found at the scene may be useful to an analyst when interpreting data. This information may be obtained from scene reports, officer narratives, sample inventories, lab processing notes, or other records of the agency responsible for the investigation of the suspected laboratory. If this information is included in the crime laboratory report, it must be referenced accordingly, and the analyst should be careful to distinguish what information they have firsthand knowledge of and what information is based on the reports of others. If the analyst's interpretation is based solely on a published literature report or other reference, the reference must be cited in the notes.

An analyst trained in the analysis of clandestine laboratories will have sufficient expertise to identify both organic and inorganic materials, including chemicals that may be important in other forensic disciplines. An analyst may note or report the presence of such chemicals, but may not write conclusions regarding their significance in his/her report unless he/she is proficient in the appropriate forensic discipline. In all instances, a scientist may testify regarding legitimate or common knowledge use of chemicals identified.

26.5.2 REPORTING

The written report should accurately and clearly convey to the reader the results of the analysis of the evidence submitted for examination. The report language should be accurate, clear, and understandable.

Seized drugs conclusively identified will be reported. Other substances that an analyst identifies and uses to report a synthesis route or method of manufacture will also be reported. Descriptive words and phrases such as "acidic, "basic", and "solvent" may be included in the report even if sufficient testing was not performed to actually determine which acid, base, or solvent is present.

Analysts are allowed discretion in the organization and detailed structure of their reports.

A report will contain the following information:

A description of evidence that was received;

- This section should include the mass or volume measurements or estimates of the items;
- This section should include any reference to the pH of the items;

Procedures

- A list of analytical methods and instrumentation used in the case, often including abbreviations or acronyms for future reference or to ease reading;

Results:

- The relevant chemicals that were identified in each item analyzed;
- Items that were not analyzed, these can be delineated in the description section instead;
- Items that contained no seized drugs, by-products from the synthesis of seized drugs, precursors or essential chemicals used in the synthesis of seized drugs;

Discussion:

- Opinions and interpretations as to the significance of the items examined, these can be delineated in the conclusion section instead;
- A brief background as to the manufacturing processes relevant to the case may be included;

Conclusion:

- A brief and clear overall conclusion of whether items submitted in this case are consistent with the manufacture of a drug of abuse, which manufacturing method(s) are indicated by the data, and any other information deemed relevant by the analyst.
- Conclusions as to estimating yield or production capacity, progress in the manufacturing process, or other conclusions based on items reportedly found at the scene and/or the scientist's analytical results may also be included. Assumptions that an analyst makes when reporting such conclusions must be clearly indicated.

The results, discussion and conclusion may be combined if appropriate.

All reports will also contain:

- Clear and understandable definitions of any technical terms, abbreviations and slang used in the report;
- The purpose of the examination;

Additional information regarding clandestine laboratory interpretation can be found in the Clandestine Laboratory Training Manual.

26.6 QUALITY ASSURANCE

For technical review, the ideal situation is one where the reviewing analyst has both the individual instrumental proficiencies as well as Clandestine Laboratory proficiency necessary to complete the review. In situations where the reviewer is not proficient in a particular instrumental technique, another scientist proficient in the technique may provide review to ensure the instrumental results are correct. The Clandestine Laboratory reviewer may then be allowed to use the results of this review in the overall review of the case.

Instrument calibration and maintenance will be performed on a regular basis, as outlined in each instruments' technical procedures. Calibration reports and maintenance are documented and can be found with the respective instruments.

Quality check results are to be evaluated. If the results are not adequate for the instrument, equipment or technique, then the instrument, equipment or technique is not to be used until appropriate action steps are taken to correct the problem. A repeated quality check that yields satisfactory results deems the instrument, equipment or technique appropriate for use.

It is essential to ensure that data collected by any analytical method is not subject to confusion or misinterpretation resulting from contamination of the case sample.

Practices to be used to prevent contamination of evidence must be employed at all times. Work areas should be kept orderly and cleaned regularly. No more than one item at a time of ~~controlled substance~~ evidence should be open in the scientist's work area. Reusable utensils or glassware should be thoroughly cleaned prior to each use. Disposable glassware and utensils will be used only once and then discarded. Appropriate blanks, including blanks of extraction, must be performed for all analyses in accordance with the technical procedures. Auto-sampler vials should be clearly marked and securely closed.

26.7 SAFETY

It is imperative that analysts take appropriate precautions during the analysis of clandestine laboratory evidence to handle these materials safely. Evidence from these cases contains unknown materials that may present explosive, flammable, contact, and/or inhalation hazards. Inadequately packaged samples need to be repackaged and defective or damaged containers replaced.

In order to be aware of possible hazards to which the analyst may be exposed, it is beneficial to obtain as much information about the suspected lab as possible, prior to analysis. This information can come from investigators, the crime scene report, and the individual who collected the samples. It is advisable to obtain a sample inventory list, which often contains observations of the individual obtaining the samples, such as the sample pH, color, volatility, and any identifying labels on the containers. However, containers at the scene may have been mislabeled. Scene inventories, recipes and other documentation relating to synthesis found at the scene may also be useful in determining what the suspected clandestine chemist was attempting to do.

PART FOUR: EXPLOSIVES AND IGNITABLE LIQUIDS

27 EXPLOSIVES ANALYSIS

27.1 INTRODUCTION

Explosives analysis entails the identification of the explosives removed from an explosive device and the examination of bomb fragments and debris recovered from the scene of an explosion. The analysis ranges from the examination of simple inorganic materials to highly complex mixtures of inorganic and organic materials. Explosive devices may range from a cardboard tube filled with black powder to a highly sophisticated device controlled by electronic circuitry and filled with high explosives such as C-4, RDX or other materials, and any combination in between.

Often bomb fragments and explosion site debris will be examined to not only attempt to identify the explosives used but also for the presence of wires, circuitry, batteries, and any other material that may provide information on the nature of the device used. Other times materials will be examined to determine if they were incendiary-type devices used to initiate a fire or cause damage to a specific object. An example would be the use of Thermite to damage a car or gain entry to a metal container.

27.2 ADVANTAGES AND LIMITATIONS

Explosive and/or pyrotechnic analysis is able to provide analytical information which may be helpful in determination of material(s) used, or in reconstruction of a device. However, the large number of available materials (both commercial and homemade) as well as possible combinations and reactivity of these materials may make determination difficult. Also, due to the nature of explosive and pyrotechnic materials, residue remaining after detonation may be minimal.

Due to the variable nature of the evidence received in analysis casework, a single approach or set of methods cannot adequately address all possible contingencies. The technical procedures in this document are therefore understood to be flexible to the requirements of each particular case.

27.3 APPARATUS AND EQUIPMENT

Explosives analysis utilizes a combination of analytical techniques. The instrumentation and techniques which are available to the scientist for explosives evidence are discussed in detail in Part Two of this manual, "Instruments & Techniques." Every laboratory conducting explosives analysis should house a reference collection containing explosives that the laboratory is likely to encounter, their constituents, explosive reaction productions, and chemicals used in the manufacturing of explosives.

27.4 PROCEDURE

27.4.1 CASE APPROACH

A case approach should be developed based on the form of evidence received, the amount of material present, and the specific questions to be addressed by the analysis. Any information about the source of materials (pre- or post-blast, locations from which they were recovered, environmental conditions, etc.) may be considered. Case approach may evolve during analysis as new data is generated or new information is received.

Observe the material visually and under the stereoscope:

- If it appears to be of uniform consistency, size, color, and texture, testing of any portion of this sample is adequate.
- If it appears to have a mixture of materials; for example: spheres, discs, granular chunks, etc., segregating the different types and testing each type individually may be in order.

- If it contains fragments from the scene of an explosion, one may see different colors, shapes, and forms of residues, as well as unexploded explosive. One may want to test these different areas separately prior to doing any type of wash of the material.

Examination of unexploded material:

- Stereomicroscopy will provide an idea of the physical nature of the material; e.g., pure crystalline, amorphous, mixture.
- Particle-picking may allow separation of the components of a mixture.
- If there is a sufficient amount of sample available, an ignition test may be in order to see if the material burns, flashes, or doesn't burn.

Examination of exploded material and fragments:

- Stereomicroscopy will permit the examination of items for the presence of unreacted explosive and explosive reaction products.
- Particle picking will permit characterization and the identification of these materials.
- If there are no visible particles, a solvent wash, such as an acetone wash (to isolate organic explosives) followed by a water wash (to isolate inorganic explosives), will facilitate subsequent tests for explosives and their reaction products.
- Physical examination of the fragments, possibly including stereomicroscopy and scanning electron microscopy, may provide information on the nature of the device and components that were used.

While the visual examination and burn test may be suggestive of an explosive, it is necessary to use additional analytical techniques to identify the explosive compound itself or its key constituents. The key constituents are highlighted for each applicable type of explosive in the charts in Appendix F: Minimum Analysis Requirements for Identification of Intact Explosives and Appendix G: Explosives and Their Post-Blast Residues.

If particulate matter is available, utilize suitable analytical methods to identify the components. An attempt should be made to identify the particulate matter prior to moving on to extractions. In the absence of particulate matter or if the analyses of the particulate matter did not provide sufficient information, extractions of the debris should be made. Normally, aqueous extracts would be analyzed by CE, LC, spot tests, microcrystalline tests, and/or flame tests. Evaporated aqueous extracts are suitable for FTIR, EDX/XRF, Raman, and/or PLM. Solvent extracts are normally analyzed by GC/MS, GC, FTIR, and/or TLC.

27.4.2 ANALYSIS

Good analytical practices suggest that multiple techniques be employed in forensic explosive identification and that supporting analytical data be available for review.

For the purposes of this document, techniques for the analysis of explosive samples may be broken down into three categories:

- Those that provide significant molecular structural and/or elemental information.
- Those that provide limited molecular structural and/or elemental information yet are highly discriminating.
- Those that are informative but do not fall in either of the other categories.

Table 1: Categories of Analytical Techniques

Category 1	Category 2	Category 3
Energy Dispersive X-Ray Analyzer (EDX)	Capillary Electrophoresis (CE)	Burn Test
Gas Chromatography/Mass Spectrometry (GC/MS)	Gas Chromatography (GC)	Flame Test
Infrared Spectroscopy (IR)	Liquid Chromatography (LC)	Melting Point
Raman Spectroscopy	Polarizing Light Microscopy (PLM)	Spot Test
X-Ray Fluorescence (XRF)	Stereo Light Microscopy (SLM)	Bulk Morphology
Nuclear Magnetic Resonance Spectroscopy (NMR)	Thin Layer Chromatography (TLC)	
Liquid Chromatography-Mass Spectrometry (LC-MS)	Microcrystalline/Microchemical Tests	
Gas Chromatography-Infrared Spectroscopy (GC-IR)		

The conclusive identification of a single substance is accomplished by the use of at least two uncorrelated analytical techniques. Typically, at least one Category 1 test is required, which should be accompanied by one or more Category 2 techniques and/or two or more Category 3 tests. Some clarifications and exceptions to this are given below:

- Chromatographic techniques may be counted as two distinct category 3 methodologies when different stationary and/or mobile phases are employed.
- PLM may be counted as two distinct category 3 methodologies when two different identification tests are done, such as examination of the physical/optical properties plus refractive index determination
- PLM can be used as a stand-alone technique in the identification of woody particles in charcoal used as a carbon source.
- EDX and XRF are sufficient on their own to establish the presence of a particular element. These techniques are not sufficient on their own for identifying the chemical form of the element (e.g., chloride vs. chlorate).
- A cord with the visual appearance of a fuse that ignites and burns appropriately may be reported as “consistent with a fuse, sufficient to ignite many explosive materials” or a similarly qualified wording.
- When identifying inorganic ions, two techniques per ion are required.
- The formation of characteristic, diamond-shaped crystals of sulfur on recrystallization is considered a Category 2 technique.

For an analytical technique to be considered of value, the test must be considered “positive”. While “negative” tests provide useful information for ruling out the presence of a particular family of explosives, these results have limited value toward establishing the identification of an explosive substance.

27.5 INTERPRETATION AND REPORTING

The identification and reporting of explosive mixtures may include (1) a specific brand name (e.g., Pyrodex®), (2) a class of mixture (e.g., “a double-base smokeless powder”), or (3) a non-specific description (e.g., “consistent with a pyrotechnic mixture”). Appendix F lists a number of intact explosive materials and the major components of each. If a specific product brand is to be reported, all of the highlighted compounds in Appendix F must be conclusively identified, and all others must be indicated by one or more analytical methods. If a specific class of explosives is to be reported, sufficient analysis should be performed to exclude other similar classes of explosives (e.g., sulfur containing vs. sulfur-free black powder substitutes). The list in Appendix F is not all-inclusive. In some cases, the material may be a hybrid or mixture of different types or the available evidence may be sufficient to suggest the presence of explosive material but not a particular type. In these instances, the categories listed in Appendix F may not be appropriate to report.

Appendix G lists a number of explosives and some components that may be encountered in post-blast debris. Components listed for each explosive are species that are known to form or reasonably expected to form from the burning of the corresponding explosive. It is likely that not all of the listed components will be present in a given post-blast sample from a given explosive, and unburned material may be intermixed with reaction products. This list is meant to be used as a guide for interpreting post-blast residue and is not a complete list of all possible reaction products.

Compiling a list of the ions, compounds, and other materials identified is recommended. Many materials are common to more than one explosive, and at times multiple explosive materials may be mixed. Some mixtures, such as pyrotechnic mixtures from display fireworks, are variable and may contain materials not listed. For these reasons, it may be necessary to include more than a single explosive source in the conclusion. A review of the charts in Appendices F and G will help the analyst decide which explosives are possible sources.

Occasionally, requests are submitted which ask to compare samples in order to determine if they had a common origin. For example, powder recovered from a device to be compared with powder found in the possession of a suspect. In such cases, even samples that are indistinguishable cannot be said to have originated from the same source. The method is more useful for exclusion of a common origin source. If a comparison regarding sample similarity is performed, it will typically be done only between samples including intact explosive material. Chemical content, grain size, color, and morphology should all be considered in comparisons between multiple samples. The report should contain wording to accurately convey the comparison findings such as below, or other similarly qualified wording:
Example: It should be noted that since similar powders/mixtures/materials are manufactured which would be (or are) indistinguishable from the submitted evidence, an individual source cannot (or will not) be determined.
Example: Both of these materials could have originated from the same source, or another similar source.

The written report should accurately and clearly convey to the reader the items received and the results of the analytical work done on the evidence submitted for examination. The report will contain a description of the device/material analyzed, methods of analysis, materials identified, and functionality of material (as appropriate). The report language should be accurate, clear, and understandable. Any limitations to conclusions reached should be clearly specified. Identifications that are less than conclusive will be reported as such.

Example: With current methods and technologies available, a complete analysis of some materials may not be possible. In some samples, there may be components that cannot be detected or identified.

27.6 **QUALITY ASSURANCE**

Instrument calibration and maintenance will be performed on a regular basis, as outlined in each instruments' technical procedure. Calibration reports and maintenance are documented and can be found with the respective instruments.

Quality check results will be evaluated. If the results are not adequate for the instrument, equipment, or technique, then the instrument, equipment, or technique is not to be used until appropriate action steps are taken to correct the problem. A repeated quality check that yields satisfactory results deems the instrument, equipment, or technique appropriate for use.

It is essential to ensure that data collected by any analytical method is not subject to confusion or misinterpretation resulting from contamination of the case sample.

Positive and negative controls will be performed and documented in case notes. The scientist should take care to employ proper methods of cleaning tools, laboratory equipment, and work areas between samples in order to prevent contamination.

27.7 SAFETY

When performing analyses on suspected explosives, explosive residue, and explosive debris, the safety of the examiner and other lab personnel should be the primary consideration. Once a package is opened, if there are any doubts as to the safety of the item, do not hesitate to call the local bomb squad for advice.

Laboratory personnel should be aware that if the presence of any explosives or explosives residue in any of the submitted items is detected, then the submitting Agency must make arrangements to collect the evidence in person.

Safety precautions for each analytical technique are outlined in the appropriate technical procedure manuals.

28 IGNITABLE LIQUID RESIDUES ANALYSIS

28.1 INTRODUCTION

Analysis and interpretation of data associated with ignitable liquid residues in fire debris and non-fire debris related matrices in the WSP Crime Laboratory are based on current methods in ASTM standards.

28.1.1 TERMS

Comparison Sample
Comparison samples, which are also referred to by the term “control samples”, are provided by the investigator. Comparison samples may include, but are not limited to, substrate material collected at the scene, material of the same composition taken from nearby the item sampled, packaging material, or absorbent material such as commercial kitty litter or other specialty absorbent products used in the collection of samples, and not suspected of containing ignitable liquid. The purpose of a comparison sample is to provide information on what compounds arise from the comparison sample which could influence data interpretation of the sample. Comparison samples do not constitute a preparation blank and should be treated as an evidentiary item.

Batch
A group of samples extracted using the same analytical procedure, reagents, physical manipulations (such as being heated together in the oven) and within the same time period.

Preparation Blank
An analytical control consisting of all reagents and solvents that is carried through the entire analytical procedure. The preparation blank is used to define the level of laboratory background contamination. Once the preparation blank has been verified as uncontaminated, it does not have to be re-run if the samples are reanalyzed. At least one preparation blank per case will be analyzed.

Solvent Blank
An analytical control consisting of a solvent, usually carbon disulfide or pentane. It is used to ensure that there is no carryover on the GC and that no components are present that will interfere with analysis. It is run before each sample.

Test Mixture
A mixture of compounds that is run to evaluate the performance of the gas chromatograph. See current edition of ASTM E1618.

Reference Ignitable Liquid
An ignitable liquid, usually commercially available, used to obtain data for comparison purposes. See current edition of ASTM E1618.

Standard Accelerant Mixture (SAM)
A one to one (v/v) mixture of gasoline and diesel that is used for comparison purposes or to evaluate methods.

Syringe Blank
When conducting headspace analysis, a syringe blank is a room-air injection from the same syringe that will be used to inject the sample. The syringe blank will be performed under the same heating conditions as the sample.

28.1.2 COMPARISONS OF IGNITABLE LIQUIDS

Occasionally, requests may be made to compare samples in order to determine if they had a common origin. For example, gasoline from a gas can recovered from the scene of a fire is to be compared with gasoline found in the possession of a suspect. In such cases, even samples that are indistinguishable cannot be said to have originated from the same source. In the case of gasoline, industry practices of production, distribution and marketing make it very difficult to specifically identify the source and brand. Likewise, the same prohibitions apply to other products; not even their intended end use can be specifically identified since the same product may find use in a wide variety of applications.

Comparison work has been done involving samples of automotive gasoline (see the training manual for references) but has been found to be of limited value, as most case situations involve attempting to compare an evaporated sample in a fire matrix to a fresh sample of ignitable liquid. There have been cases where this has been possible due to the unique circumstances of that particular case, such as having a particular odd contaminant in both the liquid and the residue from the scene, but this situation is rare. The method is more useful for exclusion of a common origin source.

28.2 **ADVANTAGES AND LIMITATIONS**

Gas chromatographs are used in the characterization of unknowns based on retention time and column selectivity. Total ion chromatograms (TICs) can be used to evaluate patterns or individual peaks. MSD adds the dimension of producing structural information from individual peaks in the TIC.

Instrument library searches can be done with mass spectra generated by the GC/MSD.

28.3 **APPARATUS AND EQUIPMENT**

A gas chromatograph must have an injection port or means of introducing a sample to a chamber (oven) which houses a column capable of separating compounds of interest. For gas chromatography (GC), the sample is injected through a heated zone (to volatilize the sample) onto a column (usually fused silica with a liquid stationary phase coated or bonded to the inner wall) and eluted using a carrier gas (He, H_2, etc.) as the mobile phase. Separated components of the mixture are detected, as they elute, by mass selective detectors (MSD) or similar instrument for that purpose.

An autosampler designed to run sequential samples without an operator present is helpful, but not necessary, for GC/MS analysis.

Reagent grade solvents or better should be used for extractions and dilutions.

A collection of disposable glass vials with caps will be maintained.

Various syringes (sized from 0.01 microliters to 10 microliters, and 1-5 milliliter gas syringes) will be used if no autosampler is present on the system.

The selected carrier gas must be of sufficient purity to meet manufacturer’s suggested specifications and must not interfere with the quality of the analysis. Carrier gas recommendations and information about the purity of the employed carrier gas will be documented in the instrument/hydrogen generator manuals and log books as appropriate. If provided by the gas supplier, certificates of quality for bottled gas will be maintained in the instrument records.

Paint cans, nylon or polyethylene bags designed for flammables analysis, and carbon strips are needed for sample preparation.

28.4 **PROCEDURE**

28.4.1 <u>CASE APPROACH</u>

During the initial inventory phase, the scientist will carry out a visual inspection of the containment of the sample to ensure that there has been no damage that would affect the analysis, noting the general condition of the container and any areas of concern in their notes. They will also briefly inspect the contents to determine the nature of the evidence, any additional forensic evidence present that might require coordination with another analysis area, and to determine which analytical techniques or combinations of techniques will be employed in the evidence analysis scheme to meet the needs of the submitting agency.

A variety of procedures for the isolation of materials from a fire debris sample may be employed, depending on the circumstances of the case and the evidence material. Selection of the appropriate technique or combination of techniques will depend on the analytical situation.

28.4.2 HEADSPACE

Headspace may be used when the sample is suspected of containing the light range ignitable liquids (including oxygenated products), and most useful for highly concentrated light to medium range ignitable liquids. Headspace can be run heated or at room temperature. Samples generated from room temperature headspace should be evaluated with caution and may need to be followed by a heated preparation. This technique is the least sensitive of the sampling techniques and may not detect volatile materials in quantities of less than 10 µL.

Refer to the current edition of ASTM E1388.

For metal cans, a small hole is made in the lid, which is then covered with tape. For vapor-tight plastic fire debris bags, this is not necessary. If the sample is to be heated it should be placed in the oven for an amount of time appropriate for the type and nature of the sample. A gas tight syringe is inserted into the tape-covered hole of the lid or into the vapor-tight bag and a vapor sample is removed from the headspace of the original container. The containers are then resealed appropriately to prevent further vapor loss. The vapor sample is injected into the injection port of the GC/MS. The size of the vapor sample depends on the concentration of analytes. Typical injection volumes range from 10 uL to 500 uL.

Ensure that the instrument has been through a syringe blank run prior to sample injection and between sample injections if any peaks were present in previous runs. As part of this process the syringe should be heated in a manner similar to the samples to prevent condensation of warmer vapors from the sample. These blanks must be satisfactory before injecting samples.

28.4.3 PASSIVE ADSORPTION ELUTION (PAE)

Passive Adsorption Elution (PAE) involves adsorption of an ignitable liquid residue (ILR) from a sample matrix onto an adsorbent material followed by elution of the absorbed ILR from the adsorbent material. Refer to the current edition of ASTM E1412.

Volatile hydrocarbons that are present in the evidence sample are adsorbed by activated charcoal. This is accomplished by "suspending" a charcoal strip (Albrayco®) or other appropriate carbon adsorption package in the headspace above the fire debris sample. If any volatile hydrocarbon materials are present in the headspace they will be adsorbed onto the charcoal strip. The strip is then eluted with an appropriate extraction solvent. Typically 100 to 1000 µL of solvent are used to elute any adsorbed materials from the charcoal strip (C-strip). An additional strip can be added if both room temperature and heated headspace are to be analyzed or if duplicate analysis is necessary. A preparation blank must be analyzed at the same time as the casework samples. Other adsorbent material may also be used for this process.

PAE is conducted by adding a charcoal strip to a can or other appropriate container. The container is then resealed and the charcoal strip is allowed to adsorb the volatile headspace components while the sample is heated as per current edition of ASTM E1412. The C-strip, either whole or split in half, is then removed and placed in a labeled glass vial. Carbon disulfide or other appropriate solvent to the sample is added to elute components from the C-strip and the subsequent solution is then analyzed by GC/MS.

A preparation blank will be prepared in accordance with Blanks and Standards section of the current edition of ASTM E1412. Blanks will be run before all samples to ensure the instrument and injection system are clean and is free of any interfering compounds. This blank may be a solvent blank or the preparation blank. Printouts of the TIC and mass spectra are produced and used for comparisons.

Precautions will be taken to prevent loss, cross-transfer or contamination of the evidence during heating by securing cans with clips, placing cans into heat-sealable bags designed for fire debris or have been found to be free of interfering components, or other techniques. The method employed for preservation of the evidence during heating will be documented in the notes.

28.4.4 SOLVENT EXTRACTION (SE)

Solvent extractions (SEs) using carbon disulfide, pentane, methylene chloride or diethyl ether can be used to differentiate between certain heavy range products in the petroleum distillates class, such as kerosene and diesel. It is often hard to classify a sample by PAE that contains a heavy range product due to fire exposure, weathering and/or fractionalization during sample preparation. When it is necessary to further classify an ignitable liquid that falls in the heavy range of the petroleum distillates class and adsorption-elution does not provide clear results, solvent extractions will be run.

There are instances when the nature of the debris or residue suggests that solvent extraction (SE) would be appropriate. A few examples of when SE would be effective are distinguishing between kerosene (terminating at or near C16) and diesel fuel (terminating at or near C23); with extremely porous substrate materials, with intricate capillary spaces; with an ILR expected to have a low heat transfer coefficient; and with aqueous solutions. Solvent extraction is not as effective for wet debris and another analysis method may be more effective for this type of matrix. See current edition of ASTM E1386.

If a portion of the total evidence sample is to be extracted with solvent, the notes and report will reflect which portion was subjected to solvent extraction. The material extracted with solvent will be packaged separately as a sub-item and will be returned to the submitting agency.

28.4.5 SOLVENT WASH (SW)

Solvent washes (SWs) can be used for non-porous materials especially when visible liquid droplets can be seen on a non-porous surface (such as pieces of glass from a suspected Molotov cocktail). The prep blank will be prepared using the same volume of solvent that was used for the SW. The solvent wash can be filtered if necessary. The wash and preparation blank can be evaporated as described in the current edition of ASTM E1386.

28.4.6 SOLVENT DILUTION (SD)

Liquid samples may be dissolved in an appropriate amount of carbon disulfide or other suitable solvent for the sample and directly analyzed by GC/MS. For hand injections in GC/MS, micro injections of 0.01 or 0.02 uL can be used without dilution.

28.4.7 IGNITION TESTING

Add a few drops of the sample liquid to glass wool on a shallow evaporating dish or other glass vessel. Ignite a flame source and move it towards the liquid. Observe the flame color and flame behavior of the sample if it ignites and record the results.

28.4.8 COMPARISON SAMPLES

Comparison samples should be analyzed using the same techniques used for the evidentiary samples. Many comparison samples are unburned or burned to a lesser degree than the evidentiary samples. These comparison samples can be analyzed in their unburned state and then burned/pyrolyzed and analyzed. If enough material is present in the comparison sample, the analyst may consider splitting the comparison sample in half. Half of the sample can be analyzed as unburned and the other half analyzed as a burned/pyrolyzed sample. A propane torch or other appropriate device can be used to burn/pyrolyze the comparison sample.

If the comparison sample is split, the documentation of this new item of evidence will comply with the requirements outlined in the QOM, Evidence Management along with the "Parents/New Parent" section of the LIMS Manual.

28.4.9 PRESERVATION OF EXTRACTS
On completion of the analysis, the PAE C-strips (or the unanalyzed half C-Strips), Solvent Washes, and Solvent Extracts and their respective prep blanks are to be returned to the submitting agency with the original evidence, as a separate evidence item. A portion of the extract will be adsorbed onto an adsorption medium and stored in a suitable container for preservation per current edition of ASTM E2451. The documentation of this new item of evidence will comply with the requirements outlined in the QOM Evidence Management along with the "Parents/New Parent" section of the LIMS Manual. The prep blank and sample C-strips produced for a proficiency test may be packaged in the container with submitted evidence and a new item in LIMS does not need to be created. The notes will reflect the disposition of the C-strips.

28.4.10 PACKAGING MATERIAL EVALUATIONS
Investigators may request the evaluation of a can or other packaging material to ensure the packaging material is free from contaminants or other interfering compounds. These items will be submitted and analyzed as a normal case.

28.4.11 DATA ARCHIVING
All generated data will be printed out and maintained in the case file. Raw data will be preserved using a write-once Compact Disk Recordable (CDR) or Digital Versatile Disk Recordable (DVD-R) and will be retained in the case file. The case number, date and initials will be documented on an area of the disk that does not store data. Generally this involves writing this information on the clear, inner ring of the disk which will be maintained in the case file.

28.5 **INTERPRETATION AND REPORTING**

28.5.1 INTERPRETATION

Ignitable liquid detection and identification using gas chromatographic data is based upon the comparison of the exhibit chromatogram with those of reference ignitable liquids materials obtained under similar analytical conditions. This technique, which is referred to as pattern recognition, uses visual inspection of the exhibit chromatogram to compare the number, position and relative peak heights of the components present with the number, position and relative peak heights of the components present in chromatograms of reference ignitable liquid materials. When gas chromatograph/mass selectivity detector (GC/MSD) is used, the additional dimension of mass spectral data is compared to reference materials of the compounds as part of the comparison process. Use the guidelines presented in the most recent edition of ASTM E1618, as applicable to the case.

Macro programs can be created to extract ion data for inclusion in the case file, but must clearly specify how the data has been collected.

If a macro is to be used to extract ion data, it will contain at a minimum the following ion extracts in the order listed below.

Alkane Profile: Extracted Ions 43, 57, 71, and 85
Aromatic Profile: Extracted Ions 91,105, and 119
Alkene/Cycloalkane Profile: Extracted Ions 55, 69, 82, and 83
Naphthalene Profile: Extracted Ions 128, 142, and 156
Indane Profile: Extracted Ions 117, 131, 145 and 159
Styrene/Methylstyrene Profile: Extracted Ions 104 and 118
Terpene Profile: Extracted Ions 93 and 136

Naphthenic/Paraffinic Profile: Extracted Ion 83
Toluene Profile: Extracted Ion 92
C2 Benzenes Profile: Extracted Ion 106
C3 Benzenes Profile: Extracted Ion 120
C4 Benzenes Profile: Extracted Ion 134
Naphthalene Profile: Extracted Ion 128
2- and 1-Methylnaphthalenes Profiles: Extracted Ion 142
Styrene Profile: Extracted Ion 104

Additional macros may be included in the case file.

Light aromatic products consisting of single or few components, major chromatographic peaks of normal alkane products, and all major oxygenated compounds as well as any other single component ignitable liquids will be evaluated by both GC retention time and mass spectral identification. Reference materials used to compare retention time and/or mass spectral data will be maintained in the case file.

28.5.2 REPORTING

See the QOM, applicable sections of the current editions of ASTM E1618 and the T/SWGFEX Standard Guide for Fire Debris Report Writing for basic report writing guidelines.

The report must include a statement as to whether an ignitable liquid was identified in the sample. The report should also contain the identity of the class that the ignitable liquid was assigned to as defined by the Ignitable Liquid Classification, and include examples from that class.

If no ignitable liquid is detected the report verbiage should be “No ignitable liquid was detected” or some other wording of similar meaning.

If no ignitable liquid can be determined due to chromatographic and spectral interferences or due to paucity of sample the report verbiage should be “No ignitable liquid was identified” or some other wording of similar meaning.

If the packaging was inappropriate for ignitable liquid samples the report should include a statement of this nature.

New items of evidence created during the course of analysis will be described in the report.

28.6 **QUALITY ASSURANCE**

See the GC/MSD section for system maintenance and calibration, as well as the instrument log books.

Test mixtures used in GC/MSD analysis shall comply with the current edition of ASTM E1618, as applicable.

The manufacturer and lot number of the carbon strips and reagents used in analysis will be recorded in the case notes.

Solvent purity will be checked by evaporating to at least twice the extent used in the analysis. Mixtures of solvent and internal standards will be checked for purity after preparation by evaporating to at least twice the extent used in the analysis. The resultant solution will be analyzed on the GC/MS and documentation of this reagent purity check will be maintained. Any compounds present in the solvent and/or internal standard which could interfere with the analysis and interpretation of data will result in the solvent and/or internal standard being deemed inappropriate for use in casework.

A new lot of charcoal strips will be evaluated to ensure effectiveness prior to use. A preparation blank and a sample of approximately 25 µl of SAM on a Kimwipe or paper towel in a lab gallon can or other volatile appropriate packaging will be evaluated using the PAE method. If the charcoal strips are deemed appropriate for use in case work the generated data will be kept with the quality assurance data provided by the manufacturer for the charcoal strips.

Compressed gas cylinders of carrier gas are usually certified down to 10% of their original pressure. At pressures below 10%, higher levels of water, organics, or other contaminants may be present which could impact the quality of testing. Compressed cylinders should be replaced when the pressure reaches 10% of their original pressure.

Thermometers used to monitor oven temperatures will be checked annually against a NIST traceable thermometer or per Manufacturer's recommendation. The thermometer being checked and the NIST traceable thermometer will be placed in the oven and allowed to equilibrate for a minimum of ten minutes. The temperature should agree within 1° C or the thermometer will be taken out of service. A record of this check will be documented in a thermometer log book. A label will be affixed to the thermometer indicating the date of check and the date the next check is due.

28.7 **SAFETY**

A minimal amount of ignitable liquid references should be stored in an explosion proof refrigerator, freezer or appropriate flammables storage cabinet.

Ignition testing, using a minimal amount of sample, and burning/pyrolyzing of comparison samples should be performed in a functioning fume hood. The flame should be completely extinguished and the glass wool and vessel cooled completely before disposal.

The analyst should have an understanding of the hazards associated with the solvents and compounds of interest in ignitable liquid residue analysis. Although a traditionally common approach, it is no longer recommended to smell the evidence as a routine preliminary step in analysis due to the carcinogenic and unhealthy chemicals present in fire debris samples. Solvent extractions, dilutions, and washes will be carried out in a fume hood.

PART FIVE: IMPRESSIONS

29 IMPRESSIONS

29.1 INTRODUCTION

Impression examinations are typically of 3 categories: direct, investigative, and class comparisons. A direct comparison is where there is a comparison between a questioned impression and an object. Shoes are the most commonly submitted evidence for this category but there are numerous other items which have been encountered such as tires, tools, fabric and cordage.

An investigative type of examination is when the lab is asked to find the make and model of a shoe or tire from a questioned impression. There are also times in which the lab is asked to attempt to determine what object, or type of object, could have made a questioned impression. These examinations may assist the investigation by refuting or including an object as being a possibility of making the questioned impression.

A class comparison is when the lab is asked to perform a comparison between a questioned impression and an object which the agency believes to be similar to what was used/encountered at that crime scene. This object is not specifically associated to the crime scene and is only submitted as a reference for comparison of class characteristics.

29.2 ADVANTAGES AND LIMITATIONS

Direct comparison conclusions from an examination may provide the submitting agency with information about whether an item was the source, or not the source, or information about the inclusion or exclusion of a particular item with a crime scene.

Investigative comparison conclusions from an examination may provide the submitting agency with information about a possible shoe/tire/object that made a questioned impression.

Class comparison conclusions from an examination may either include or exclude an item which may assist with the context of the scene and/or help support case reconstruction efforts.

Additional information that may be provided from an examination is a determination of order of deposition of overlapping questioned impressions, how the questioned impression was made, the direction of travel, the brand or manufacture of the footwear or tire, possible size range of the footwear, or who was last to operate a motor vehicle

Some situations that may create limited potential information from an examination are the substrate features, quality of the original questioned impressions, and/or the method of documentation and collection. Questioned impressions may also require an interim enhancement technique (i.e. impressions made in blood) during the examination process.

29.3 APPARATUS AND EQUIPMENT

Stereomicroscopes and rolling microscopes along with basic laboratory supplies and equipment are needed for examination of impression evidence.

29.4 PROCEDURE

29.4.1 ANALYSIS

Start with a clean work area and examination paper for each item examined.

All examinations, relevant observations, and results are to be documented.

Direct Comparisons

It is recommended that one begins with the questioned impression and/or questioned evidence and at a minimum record a general description of the questioned impression before opening up the item to be compared to. At this point a visual examination of the questioned impressions and the submitted item for comparison and/or photographs can be conducted to evaluate if there are or are not general similarities between the two. This screen may quickly eliminate the known items as a source of the questioned impressions and limit the necessary scope of the examination.

The procedures that follow need not be performed in the given order.

The analyst needs to be aware of and record possible limitations in questioned impressions and/or questioned evidence that can be due to substrate features, quality of the impressions, and the method of collection. Also determine if the questioned impressions are positive or negative impressions and if they are 2-D or 3-D.

If needed, the questioned impression and/or questioned evidence may be digitally imaged and digitally enhanced (as with Adobe® Photoshop®) as necessary/appropriate or enhanced using wet chemical methods. Procedures for some commonly used chemical enhancements can be found at the end of these procedures (or in the appendix).

Evidence (questioned or known items) may require initial preparation prior to the examination process. Preparation may include cleaning casts, recognition and preservation of other relevant physical evidence, photographic documentation, enhancement, and printing of photographic images.

When examining casts carefully remove adhering soil and debris. Do not use sharp tools to remove clods. Remaining soil can be removed with a combination of gentle brushing and rinsing with water. This debris is to be packaged and retained with the submitted item(s) or made a new item of evidence.

The process of comparing the questioned impressions to the submitted known item is usually performed by making 1:1 test impressions (exemplars) of the submitted items and comparing these exemplars to the questioned impressions. Visual reference and additional comparison to the known item is conducted to confirm corresponding individual markings, and wear markings.

Before making exemplars of the known object one should be aware of any embedded debris or adhering material which may be individualizing characteristics. However, it may be necessary to remove loosely adhering debris from the surface of the outsole before making the exemplars. This debris is to be packaged and retained with the submitted item(s) or made a new item of evidence.

One may need to consider how the questioned impression was deposited (walking, running, kicking) as this may dictate how the exemplars are to be made and/or if test experiments to create additional exemplars need to be conducted.

Other 1:1 exemplars may be employed in the examination as necessary which may include; tape lifts of particular areas of the outsole or tire tread, inking, carbon paper tracings, clay, and silicone spray and magna powder impressions.

Sufficient exemplars need to be made to ensure the reproducibility of the individualizing characteristics present in the known item.

If the class characteristics of the questioned shoeprint or tire track and the reference shoe or tire are the same, then a comparison of their individual marks (accidental characteristics) is done. Accidental characteristics are cuts, tears, wear marks, randomly embedded objects, and some flaws acquired during or after manufacturing.

Tire impressions are also compared to submitted tires by the exemplar overlay method; however, because the complete length of a tire impression that represents one full revolution of the tire is many times longer than the length of a shoeprint, a different approach to the exemplar overlay method is required. First, the examiner must determine what part of the sequence of pitch lengths (length of a repeating unit in a design pattern) is represented in the question impression. Determining the exact sequence of differing pitch lengths allows the analyst to narrow down the area of comparison.

The results of the examination and comparison of the questioned impression(s) and submitted known/suspected source will be reflected in the notes. The documentation will include but is not limited to notation of class characteristics, individual characteristics, physical size (dimensions vs. size), wear, superimposability of the patterns/designs mirror images, any damage to the item, and any adhering debris, deposits and/or stains. Where possible, the identified source or possibilities are to be included as well.

Investigative Comparisons

For investigative comparisons that only involve a suspected impression (footwear and/or tire) a reference database, the internet, and/or other appropriate resources/references (retail stores) may be used.

For questioned footwear impressions one can search the internet, appropriate resources/references such as the SWGTREAD scientific forum, or submit to the Seattle Crime Laboratory for a search of the foster+freeman Shoeprint Image Capture and Retrieval (SICAR®) casework management system. Compare the questioned impression to a possible tread pattern for class characteristic similarities.

If an outsole was found on the internet or other appropriate resources/references one must include the website, or appropriate reference along with images of the outsole design.

For questioned tire impressions one can use the internet, and/or the Tread Design Guides (A Bennett Garfield Publication) which is a publication that contains tire information and tire tread designs for passenger, light truck, and other vehicles. If a tire was found on the internet or by use of the Tread Design Guides one must include the website reference, a copy of the pertinent sections of the Tread Design Guides, and images of the tire tread design.

For questioned impressions that are not shoes or tires in which an object was found to be a possible source of the questioned impression, one must include all of the appropriate references along with sufficient images to support the conclusions.

Class Comparisons

For class comparisons of an item submitted that is not an item involved in the crime, follow the direct comparison procedures.

29.4.2 NOTE TAKING

Limitations of the evidence that affect the examination are to be noted and/or explained; this can include such issues as no scale in submitted photographs/digital images, photos taken at an angle, incorrect lighting during photo documentation, and blurry or smudged lifted impressions.

A complete description of the submitted footwear is necessary including: the condition, manufacturing information, general design features, outsole wear pattern, adhering debris, labeling, stains, and any other significant features or trace evidence.

A complete description of the submitted tires is necessary and needs to include brand name, manufacturer, size, condition, general design features, and the DOT serial number system.

The notes must include supportive reasoning for the inclusion/exclusion of submitted footwear, tires, or fabric as a source of the questioned impression(s). This is best represented by a sketch and/or copy of the questioned impression and the exemplars that are both marked with the locations of the corresponding individualizing characteristics, wear patterns, or what is dissimilar between the impressions and submitted footwear or tires.

29.4.3 PACKAGING

The nature and condition of the items submitted will dictate the packaging. A different type of packaging method may be necessary after examination in order to retain the integrity of the submitted item(s).

Evidence created due to the examination may be sealed and re-packaged with the submitted item or may be made a new item of evidence depending on what was removed and/or collected.

Do not package casts in plastic.

29.5 INTERPRETATION AND REPORTING

It may be necessary and/or useful to examine crime scene documentation, especially photos, to help establish and/or support observations and conclusions about the significance of the questioned impressions, their relationship to each other and possibly other physical evidence, and addressing specific investigative questions.

The individualizing characteristics must be of sufficient quantity and/or uniqueness and correspond in location, shape, and dimensions between the questioned impression and the submitted known item to support the statement and opinion that the particular submitted footwear, tire, or clothing item made the questioned impression to the exclusion of all others.

Due to varying circumstances, not all individual characteristics will reproduce in every impression. Therefore, the absence of an individual characteristic is not a basis for elimination and does not preclude identification. The overall features of the questioned impression must be taken into consideration and compared to the exemplars produced and the physical known item in determining the strength of the conclusions that can or cannot be made.

29.5.1 DIRECT COMPARISONS REPORTING

Conclusions in which a questioned impression and a known item were submitted for comparison will be expressed based on the Scientific Working Group for Shoeprint and Tire Tread Evidence (SWGTREAD) at the end of this manual, includes “Lacks sufficient detail, Exclusion, Indications of non-association, Limited association of class characteristics, Association of class characteristics, High degree of association, and Identification.”

The same conclusions (with appropriate modifications) can be used for items that are not shoes or tires.

29.5.2 INVESTIGATIVE COMPARISONS REPORTING

For investigative comparisons in which an item (footwear, tire, or other) was found which could have made the questioned impression a disclaimer such as “other footwear (or whatever the item is) with similar outsole designs are included in the population of possible sources” will be in the report.

29.5.3 CLASS COMPARISONS REPORTING

For a class comparison in which the object submitted is not an item involved in the crime a conclusion of "Identification" cannot be made. A disclaimer such as "this item, or another similar item, could have made the impression" will be in the report.

29.5.4 SWGTREAD RANGE OF CONCLUSIONS STANDARD FOR FOOTWEAR AND TIRE IMPRESSION EXAMINATIONS (03/2013)

The following descriptions are meant to provide context to the levels of opinions reached in footwear and tire impression comparisons. Each level may not include every variable in every case. This applies to both partial and full impressions.

Lacks sufficient detail:
No comparison was conducted: the examiner determined there were no discernible questioned footwear/tire impressions or features present. This opinion applies when there is insufficient detail to conduct any comparison.

> In the opinion of the examiner, an impression was either not present or the impression lacked sufficient detail for any comparison.

A comparison was conducted: the examiner determined that there was insufficient detail in the questioned impression for a meaningful conclusion. This opinion only applies to the known footwear or tire that was examined and does not necessarily preclude future examinations with other known footwear or tires.

> In the opinion of the examiner, the impression lacked sufficient detail for a meaningful conclusion regarding the particular known footwear outsole or tire tread.

Exclusion:
This is the highest degree of non-association expressed in footwear and tire impression examinations. Sufficient differences were noted in the comparison of class and/or randomly acquired characteristics between the questioned impression and the known footwear or tire.

> In the opinion of the examiner, the particular known footwear or tire was not the source of, and did not make, the impression.

Indications of non-association:
The questioned impression exhibits dissimilarities when compared to the known footwear or tire; however, the details or features were not sufficiently clear to permit an exclusion.

> In the opinion of the examiner, dissimilarities between the questioned impression and the known footwear or tire indicated non-association; however, the details or features were not sufficient to permit an exclusion.

Limited association of class characteristics:
Some similar class characteristics were present; however, there were significant limiting factors in the questioned impression that did not permit a stronger association between the questioned impression and the known footwear or tire. These factors may include but were not limited to: insufficient detail, lack of scale, improper position of scale, improper photographic techniques, distortion or significant lengths of time between the date of the occurrence and when the footwear or tires were recovered that could account for a different degree of general wear. No confirmable differences were observed that could exclude the footwear or tire.

> In the opinion of the examiner, factors (such as those listed above) have limited the conclusion to a general association of some class characteristics. Other footwear or tires with the same class characteristics observed in the impression are included in the population of possible sources.

Association of class characteristics
The class characteristics of both design and physical size must correspond between the questioned impression and the known footwear or tire. Correspondence of general wear may also be present.

> In the opinion of the examiner, the known footwear or tire is a possible source of the questioned impression and therefore could have produced the impression. Other footwear or tires with the same class characteristics observed in the impression are included in the population of possible sources.

High degree of association
The questioned impression and known footwear or tire must correspond in the class characteristics of design, physical size, and general wear. For this degree of association there must also exist: (1) wear that, by virtue of its specific location, degree and orientation make it unusual and/or (2) one or more randomly acquired characteristics.

> In the opinion of the examiner, the characteristics observed exhibit strong associations between the questioned impression and known footwear or tire; however, the quality and/or quantity were insufficient for an identification. Other footwear or tires with the same class characteristics observed in the impression are included in the population of possible sources only if they display the same wear and/or randomly acquired characteristics observed in the questioned impression.

Identification
This is the highest degree of association expressed by a footwear and tire impression examiner. The questioned impression and the known footwear or tire share agreement of class and randomly acquired characteristics of sufficient quality and quantity.

> In the opinion of the examiner, the particular known footwear or tire was the source of, and made, the questioned impression. Another item of footwear or tire being the source of the impression is considered a practical impossibility.

29.6 QUALITY ASSURANCE

Impression evidence identifications of questioned impressions to a known will be verified by a qualified examiner in accordance with the QOM.

Reagents used for the development or enhancement of impression evidence will be checked with positive and negative controls as described in Appendix A: Reagent Preparation.

29.7 SAFETY

Take appropriate safety precautions to prevent breakage when working with casts or other fragile evidence submitted for an impressions examination.

30 SHOEPRINT IMAGE CAPTURE AND RETRIEVAL (SICAR®) EXAMINATION

30.1 INTRODUCTION

There are times that an agency submits impression evidence to see if it is possible to determine information about footwear that may have been present at a crime scene. Historically, one's experience and/or the internet have been utilized for searching for a footwear outsole with similar details as found in a questioned impression. The use of *foster+freeman*'s Shoeprint Image Capture and Retrieval (SICAR®) casework management system in conjunction with the SoleMate footwear database is an additional tool to help provide shoe information.

The SICAR® casework management system is a computer application with over 28,000 outsoles that can be searched against to provide manufacturer's shoe brand/model information. This database is used by coding information about a questioned impression; not by electronic comparison of an image of a questioned impression to the database. Coding is, in essence, adding appropriate shapes and logos seen in the questioned impression to be used for searching against the database. Information that can be generated from a search may be a record (a shoe or multiple shoes with a similar outsole design) or a group (multiple records with a similar outsole design).

30.2 ADVANTAGES AND LIMITATIONS

A SICAR® database search may be used to provide footwear brand/model information from impression evidence left at a crime scene. The use of this system may also be valuable in comparison examinations where the questioned impressions are not consistent with the Known shoes submitted. These examinations may be able to provide helpful investigative information.

30.3 APPARATUS AND EQUIPMENT

The following equipment is needed for performing brand/model searches:

- SICAR® software and manuals
- Computer data station with appropriate operating system
- High resolution digital camera
- Scanner and printer
- Internet access

30.4 PROCEDURE

30.4.1 ACCEPTANCE OF SICAR IMAGES

For a SICAR® examination (investigative examination only) there are a variety of ways to submit images to be searched.

Images may be submitted over the counter as an item of evidence. In these situations the images need to be in a sealed condition along with an accompanying RFLE. The exam code for the RFLE should be marked as SICAR® or SICAR® Search.

Images may be submitted using electronic mail as follows:

- An electronic RFLE must be filled out and attached in an email (RFLE should include agency, agency case number, agency representative with email and phone contact information along with specific notes regarding the request, if any).
- The image(s) with impression(s) that are to be searched are attached in the same email (due to server limits we recommend that the file size for all images not exceed 25 MBs).
- The email with attached RFLE and image(s) is then sent to shoesearch@wsp.wa.gov

For those that will be performing the SICAR® database search, the requirements for entering electronically submitted images (email or file transfer protocol [FTP]) are described as follows:

- Open email and print for case file
- Print RFLE
- Generate (if needed) a laboratory case number and enter information in LIMS
- Create a CD or DVD of the images
- Enter the submitted image information on the RFLE if not provided
- Respond via email to the submitting agency/individual that the request has been received, which scientist is assigned to perform the examination and that scientist's contact information

30.4.2 ANALYSIS

Impression examinations begin similar as to that found in the Impressions chapter of this manual. For searches, the necessary step to use the database consists of getting any suspected impression(s) into a digital format to be used for entry. This could be by photographing or scanning an item of evidence containing the suspected impression(s). Evidence can also be submitted as electronic formats such as compact discs and these images can be transferred directly.

The image with the questioned impression is then entered into the SICAR® system. The details of a questioned impression are coded and this information is searched against the database. Instructions for entering an image and the coding of a questioned impression are outlined in the SICAR® User Manual.

For any search that generates a record/group the shoe outsole design of this record/group should first be compared to the questioned impression. If a generated record/group is associated with multiple shoe models one should evaluate if all of these models exhibit a similar outsole design. If the SICAR® database does not include an outsole design for a particular model one can use internet searches or retail store(s) to assist in this evaluation.

Limited information in questioned impressions may also limit the amount of information available to search, or code. This may be a factor if a result is generated or not.

30.4.3 NOTE TAKING

Limitations of the submitted evidence that may affect the examination are to be noted and/or explained. The coding steps used will be printed and kept as notes in the case file. Record(s) that are found that may be possible footwear sources of an impression(s) are to be printed and kept as notes in the case file.

30.5 **INTERPRETATION AND REPORTING**

The Methods and Observations section of the report should include a disclaimer such as:

"A search of the Shoeprint Image Capture and Retrieval (SICAR®) Reference Database was used to search for records of similar outsole design(s) to the questioned footwear impression(s). SICAR® is a footwear database which contains manufacturer information including outsole patterns to aid in identifying potential make and/or models of footwear impressions recovered from scenes of crime."

Some example wording for conclusions to be used in reports are as follows:

Report wording for a negative search result:

A search of the Shoeprint Image Capture and Retrieval (SICAR®) Reference Database did not reveal any records with a similar outsole design. Although this search did not reveal any similar outsoles, the database gets periodically updated and can be searched again if needed.

If suspect shoes are obtained and a comparison between these and the questioned impression(s) is needed, please submit the shoes and original images or item of evidence.

<u>Report wording for a search resulting in one record for possible source:</u>

A search of the Shoeprint Image Capture and Retrieval (SICAR®) Reference Database revealed the Nike Revolution as a possible source of this impression or other outsoles with a similar design are included in the population of possible sources. Information about the make/model and an image of the outsole are provided below.

If suspect shoes are obtained and a comparison between these and the questioned impression(s) is needed, please submit the shoes and original images or item of evidence.

<u>Report wording for a search that resulted in several records of similar overall outsole design as the questioned impression:</u>

A search of the Shoeprint Image Capture and Retrieval (SICAR®) Reference Database revealed several makes/models of footwear as possible sources of the impression including but not limited to the following: Nike (Sweet Classic and several other models), Dickies (Marco), Dunlop (New School) and Ellesse (Kansas) or other outsoles with a similar design are included in the population of possible sources. Information about the make/model and an image of the outsole are provided below.

If suspect shoes are obtained and a comparison between these and the questioned impression(s) is needed, please submit the shoes and original images or item of evidence.

<u>Report wording for a search that resulted in a similar record that also exhibited differences from the questioned impression:</u>

A search of the Shoeprint Image Capture and Retrieval (SICAR®) Reference Database revealed the Brahma Bruiser boot to have a similar general outsole design as the impression depicted in the submitted image. However, there were also differences between the logo area of the impression and the Brahma logo on the Bruiser boot. Therefore, the source of the impressions in the submitted images is an item of footwear that shares the same general outsole design as the Brahma Bruiser, but could be of a different brand. The uppers of the footwear that made these impressions may also differ from the Brahma Bruiser.

If suspect shoes are obtained and a comparison between these and the questioned impression(s) is needed, please submit the shoes and original images or item of evidence.

<u>Example report wording for a search that did not find a result but found an outsole with similar outsole design element characteristics to the questioned impression:</u>

A search of the Shoeprint Image Capture and Retrieval (SICAR®) Reference Database did not reveal a possible source of the questioned impressions represented by the questioned impressions in the submitted images. An Asics Gel-Cumulus 15 does share design element similarity, broken squares, as to the questioned impressions.

If suspect shoes are obtained and a comparison between these and the questioned impression(s) is needed, please submit the shoes and original images or item of evidence.

Example report wording for a search resulting in numerous records but not enough information to further narrow down to a specific outsole:
Outsoles with zig zag patterns and a solid border are commonly encountered. Although common, a search of the Shoeprint Image Capture and Retrieval (SICAR®) Reference Database did not reveal an outsole as a possible source for the impressions in the submitted image due to the lack of detail in the arch area.

If suspect shoes are obtained and a comparison between these and the questioned impression(s) is needed, please submit the shoes and original images or item of evidence.

Additional report information:

Limited information in questioned impressions may also limit the amount of information available to search, or code. This may be a factor if a result is generated or not and may be information that is necessary to add to the report.

30.6 QUALITY ASSURANCE

On searches which provide a reportable brand/model:

The generated record will be compared and verified with the impression(s) in question. This verification will be noted on the record and will be part of the technical review.

On searches which do not provide a reportable brand/model:

The supervisor, or designee, will perform the review of a search that did not provide a reportable brand/model. He/she will perform a SICAR® search to determine if a reportable brand/model was possibly missed or if there may be a different coding entry which may provide success. If the search does still not provide a record this will be noted in the case file and will be part of the technical review.

30.7 SAFETY

There are no specific safety precautions associated with this category of testing.

PART SEVEN: MATERIALS

27 BOTANICALS

27.1 INTRODUCTION

Botanical examinations involve small, often incomplete fragments of leaves, woody and non-woody fibers, needles, grass, stems, thorns, weeds, flowers, tobacco, seeds, diatoms, pollen and spores which are found as associative evidence on or in clothing, vehicles, and soil.

Botanical examinations rely on the identification of unique morphological features which are often taxonomically significant to order, genus, and species.

27.2 ADVANTAGES AND LIMITATIONS

Examinations are conducted for proper characterization and comparison of botanical materials. Species identification is seldom performed with the exception of some woody material including lumber and pulpwood.

27.3 APPARATUS AND EQUIPMENT

In addition to standard laboratory supplies, microcrystalline reagents and various stains and clearing agents will be needed. A reference collection of botanical materials which may include wood and wood-pulp fibers should be available.

27.4 PROCEDURE

27.4.1 CASE APPROACH

Always be aware of the potential for additional trace materials to be present and the possibility of a physical match.

Examinations are initially conducted visually and using a stereomicroscope. Thin-sections may be prepared and mounted for examination by light microscopy in conjunction with a variety of stains, reagents, and clearing agents to help visualize internal and/or external features. Herbarium samples, identification manuals, and reference keys are often used to aid in identification. Phase contrast, fluorescence, and scanning electron microscopy (SEM) may also be used to characterize these materials.

27.4.2 ANALYSIS

Botanical materials can be recovered from practically any substrate including clothing, bodies, vehicles, carpet, etc. Recovery methods include picking, scraping, tape-lifting and in the case of pollen in dust, micro-vacuuming. Botanicals found in water can be recovered with a net or sieve. Tape lifting of botanicals is not typically recommended due to their fragile structure. The collection of botanical control specimens may involve drying and pressing techniques and simply picking of materials at the scene into paper bags or envelopes. Do not collect botanical materials in plastic containers. Use semi-permeable containers such as paper bags.

One of the first steps is to determine the sampling technique(s) to be used prior to analysis. Observe the material under the stereoscope. If it appears to be of uniform consistency, size, color and texture, then testing of any portion of this sample is adequate.

If it appears to have a mixture of materials, segregating the different types and testing each type individually may be in order. Botanical materials can be sampled from the rootlets, stems, flowering parts, bark, etc. and mounted on slides or sectioned for examination. Representative samples can be picked, scraped, or cut from specimens for examination. Mark the location of any materials removed for testing and record this information in laboratory notes with a sketch or photograph.

Specimens may be sectioned to reveal internal structure (e.g., wood, tape, needles). The majority of botanical specimens such as wood can be sectioned with a single-edged razor blade. For very small specimens the sectioning can be performed while viewing through the stereomicroscope. Transfer prepared sections to microscopes slides, cover with a coverglass, and add appropriate mounting media.

Sieving may be required when screening, segregating and separating particles such as seeds. Record the screen(s) used and the type and amount of materials recovered on each screen.

Woody Materials and Pulpwood fibers
Wood and wood products are submitted to the crime laboratory for characterization, identification and often comparison. Items may be as diverse as sections of harvested trees cut illegally, branches and limbs imbedded in vehicles or victims, wood used as weapons such as baseball bats and lumber, and microscopic traces in garden soil, charred remains, and sawdust.

Macroscopic gross features in wood can often be examined visually. Always be aware of the potential for a physical match with another fragment.

Photograph the woody material prior to removal of particles and sectioning.

Particle size is typically small and magnification with a simple hand lens or stereo-binocular microscope is necessary.

Examinations are first made for the presence of adhering evidence such as blood, fingerprints, tissue, paint, fibers, hair, etc.

If the sample is of sufficient size consider softening the wood in a beaker of boiling water prior to cutting. The wood is sufficiently softened when the wood sinks. Start by locating the end grain visually or by carefully whittling down the wood to see the growth ring orientation. Using a fresh single-edged razor blade, expose the cross-sectional view. Many wood samples can be identified by the exposed anatomy in this orientation. Next radial and tangential surfaces are located and exposed using a sharp razor blade. Cut sections as thin as possible in a single smooth motion while the wood is still moist (avoid using sawing motions). Minute fragments will need to be oriented and sectioned under the stereomicroscope. Transfer the cut sections to microscope slides. If the sections are curled upon transfer, add a small drop of water to the section and gently tease it flat. Place a cover glass onto the preparation and instill a drop or two of water along the edge of the cover glass. If you will be making examinations over a long time period, you may wish to try a mixture of equal parts ethanol and glycerin as the mounting media. Warming the slide on a hot plate will expel air bubbles and the slide will last for days. Permanent preparations of the wood sections can be made using Meltmount™ and/or Norland™ optical adhesive. The use of published identification keys and flow charts will aid in the characterization and identification of hardwood and softwood species.

Pulpwood fibers
Paper is a very common commodity and is submitted to the crime laboratory as newsprint, writing tablet paper, packaging material, blow-in cellulose insulation, napkins, paper matches, wallpaper, cardboard, etc. Proper identification is performed using the light microscope in conjunction with a variety of stains such as Graff's C stain. Softwood fibers are identified by the presence of bordered pits also called "donut cells". Hardwood fibers lack bordered pits, but instead are identified by vessel elements also called "baggy cells". The Technical Association for the Pulp and Paper Industry (TAPPI) has published specific guidelines for the examination of pulp and paper which should be consulted prior to analysis.

27.4.3 NOTE TAKING

Notes should include the source of each item, sample treatment/preparation steps to be followed, results of testing and any data from instrumental analysis.

27.4.4 PACKAGING

Precautions should be taken to prevent cross-contamination between known and questioned samples. Known and questioned samples should never be packaged together and all packaging should be sealed to prevent leakage of small items of evidence into or out of the packaging container. If the original packaging is not secure (e.g., torn paper envelope), place it in an appropriate sealable package. Seal and mark the package with initials, lab number, item number, and date.

27.5 INTERPRETATION AND REPORTING

Botanical examinations and comparisons are qualitative techniques and as such do not utilize measurements that will have a significant effect on the outcome of the analysis. Measurements are occasionally taken to document the size of particles but are not used to establish a critical value.

In cases where only questioned samples are submitted, the examiner will attempt to identify the chemical make-up or possible origin of the material in order to provide investigative information. Typical conclusionary terms include: indistinguishable, similar, cannot eliminate, inconclusive, dissimilar and exclusion. The presence and abundance of unusual or rare materials can weigh heavily on the degree of commonality in the conclusion statement and should always be addressed.

27.6 QUALITY ASSURANCE

The scientist should take care to employ proper methods of cleaning tools, laboratory equipment, and work areas between samples in order to prevent contamination.

27.7 SAFETY

The vapor from some clearing agents may be hazardous to your health which may require preparation within a fume hood.

28 BUILDING MATERIALS

28.1 INTRODUCTION

Building materials are composed of particles that cross a diversity of microanalysis sub-disciplines including paint, wood, fibers, soil/geological, botanicals and glass. This procedure will focus on engineered wood, asbestos, natural rock, Portland cement, hardened concrete, wallboard, roofing, insulation and fireproofing materials. The analytical procedures required are dictated by the type of material encountered.

28.2 ADVANTAGES AND LIMITATIONS

The examination of adhering debris on discharged bullets may help define the bullet path including impact with intervening materials. Fibrous insulation, gypsum wallboard dust, paint, glass, wood dust, etc. may transfer to a suspect's clothing and hair during a burglary. Tools found in a suspect's possession may have building materials smeared or adhering to them which may offer important clues in an investigation. Motor vehicles involved in an accident may be an excellent repository of building materials from contact with an immovable object such as a concrete barrier or wooden utility pole.

28.3 APPARATUS AND EQUIPMENT

In addition to standard laboratory supplies, microcrystalline reagents and various stains and clearing agents will be needed. A reference collection of building materials which may include concrete, cements, engineered wood, roofing, siding, insulation, asbestos, etc. is required.

28.4 PROCEDURE

28.4.1 CASE APPROACH

The potential for a physical match between known and questioned samples must be considered before selecting the method of sample collection. Analysis generally begins with appropriate non-destructive tests. If the initial tests are inconclusive or not exclusionary, the examination may proceed with additional tests that are selected on the basis of their potential for use in evaluating or discriminating the samples of interest. For any given examination, not all the techniques listed are necessarily required. Sample size, condition, and structural complexity should be considered when determining which techniques to use.

28.4.2 ANALYSIS

Building materials can be recovered from practically any substrate including clothing, bodies, vehicles, carpet, etc. Recovery methods include picking, scraping, tape-lifting and micro-vacuuming.

Observe the material under the stereo-binocular microscope. If it appears to be of uniform consistency, size, color and texture, then testing of any portion of this sample is adequate. If it appears to have a mixture of materials, segregating the different types and testing each type individually may be necessary. Representative samples can be picked, scraped, or cut from specimens for examination. Mark the location of any materials removed for testing and record this information in laboratory notes with a sketch or photograph.

Visual and stereo-binocular microscopic techniques will allow you to determine the morphology of the material and perform physical examinations and sectioning.

Polarized light microscopy is used to determine the nature of inorganic and organic materials by observation of their optical properties.

The use of various stains will give indications as to the nature of the examined material.

Microcrystalline and wet chemical testing will aid in the determination of specific ingredients (e.g., 10% HCL test for gypsum).

Scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) can generate information regarding the elemental composition of inorganic constituents in building materials. SEM can also be used to characterize the morphology of particles within samples. For SEM-EDS analysis particles can be mounted on aluminum stubs having an adhesive surface. The majority of samples will not require carbon coating; an exception may be in the examination of polymers and wood-plastic composites.

Infrared spectroscopy is useful in the analysis of building materials. Inorganic particles tend to have most of their significant infrared absorptions in the lower frequency spectral regions, and several have all of their absorptions in the region below 700 cm-1. Generally, the FTIR microscope will provide the most satisfactory results for the analysis of materials with the least amount of sample. The scientist may choose the appropriate collection parameters for analysis of the sample based on sample type or material.

28.4.3 NOTE TAKING

Follow general note taking guidelines.

28.4.4 PACKAGING

Follow general evidence packaging guidelines.

28.5 INTERPRETATION AND REPORTING

Materials examinations and comparisons are qualitative techniques and as such do not utilize measurements that will have a significant effect on the outcome of the analysis. Measurements are occasionally taken to document the size of particles but are not used to establish a critical value.

In cases where only questioned samples are submitted, the examiner will attempt to identify the chemical make-up or possible origin of the material in order to provide investigative information.

Typical conclusionary terms include indistinguishable, similar, cannot eliminate, inconclusive, dissimilar and exclusion. The presence and abundance of unusual or rare materials can weigh heavily on the degree of commonality in the conclusion statement and should always be addressed.

28.6 QUALITY ASSURANCE

Quality assurance includes the proper calibration and maintenance of the instruments/equipment used. The scientist should take care to employ proper methods of cleaning tools, laboratory equipment, and work areas between samples in order to prevent contamination.

28.7 SAFETY

Building materials that contain asbestos minerals should be examined in a ventilated hood due to potential carcinogenic properties. After a portion of a material containing asbestos is mounted on a slide or packaged in a sealed container, it may be examined out of the hood. Seal all waste containing asbestos and follow hazardous waste disposal methods.

29 DAMAGE ASSESSMENT

29.1 INTRODUCTION

There are numerous types of materials that can be submitted for damage assessment examination, but the majority of the evidence examined is of clothing/textile construction. This type of examination on clothing submitted for analysis can often determine if the damage was incident to a struggle (tearing or ripping), a strong impact, projectiles, knives, or from normal wear and tear. Mechanical damage is the form of damage usually examined; however, chemical and thermal damage are also encountered.

The experienced scientist should be able to, in many cases, determine the cause and/or manner of the damage, identify and exclude weapon/implement type(s) used, provide a probable sequence of events, if appropriate, and address other questions as necessary.

29.2 ADVANTAGES AND LIMITATIONS

Results of damage assessment examinations may substantiate and/or refute a victim's story, a suspect's alibi, or an officer's theory of how events transpired. Damage assessments may put the victim in contact with the suspect's car or may aid the investigator in determining who was driving a vehicle when an accident occurred. Damage assessments may also aid investigators in locating and/or identifying suspect weapon/implement(s).

Experiments will likely need to be conducted as part of this examination to determine if the submitted and/or suggested weapon(s) could have made the questioned damage. These experiments will utilize the questioned weapon(s) and an undamaged area of the known substrate as individual case parameters allow. Like weapon(s) and/or substrate(s) may need to be employed in the experiments if the submitted questioned evidence cannot be used. An appropriate scientific approach must be used when performing these experiments.

29.3 APPARATUS AND EQUIPMENT

Sample handling tools, packaging materials, and a variety of microscopes are needed to perform physical match examinations. A variety of weapons which are case specific will be needed for elimination and/or inclusion. Individual case circumstances will dictate additional requirements and modifications.

29.4 PROCEDURE

29.4.1 ANALYSIS

The evidence is examined macroscopically to determine if there are any damaged areas. A stereomicroscopic search may also be required to locate and/or confirm damaged areas.

During the examination process, the handling and manipulation of the evidence should be as minimal as possible, especially in the damaged areas, to as best preserve the features and surrounding conditions of the damaged area(s). Note the location, shape, and dimensions of all damaged areas. Note if the damage is recent or old where possible. Documentation by sketching and photographs is recommended.

Damage testing including substrate separations (cuts, tears, etc.) shall be conducted on like substrate composition as the questioned item with the damage. Photos and sketches of damage tests are recommended for the case notes. The article(s) containing the questioned separation(s) should also be used for the test separations as long as there is an area accessible that will not comprised the questioned separations or other evidence. This area of test separations will be marked as such by the analyst.

If a suspected weapon/implement (e.g., knife, scissors) is submitted, it should be examined using a stereomicroscope for the presence of textile fibers or other adhering trace and/or biological evidence prior to making any test cuts. If there is adhering evidence, this evidence will need to be documented and properly collected before any test cuts are executed. Consult with DNA and the Latent Prints sections when needed.

The following damage may be able to be identified on the basis of macroscopic and microscopic characteristics:

- Normal wear
- Cuts
- Tears
- Punctures/Stabs
- Snags
- Abrasions
- Burns, melting, or other thermal exposure
- Seam separations
- Chemical exposure/reaction
- Insect damage

All pertinent damaged areas are examined using a stereomicroscope. Conditions/features to note are whether or not the ends are matted together, if there is fiber melting, and whether or not there is adhering debris in the damaged areas. Debris such as charred deposits, apparent lead residues, foreign fibers, glass particles, and/or paint particles at the separation edge(s) are to be noted as necessary. This evidence may also need to be collected for separate comparisons.

The stereomicroscopic appearance of the test substrate separations is compared to that of the questioned substrate separations. The substrate ends/edges and surrounding weave/material of the damaged areas are compared for similarities/differences between the questioned damage and the experiment separations. This provides the data for what conclusions may be drawn.

Any dirt, debris, stains, and material adhering to the item may need to be removed and/or characterized or compared.

Findings of normal wear are noted, but are not typically reported unless pertinent to the case.

Depending on the case scenario, it may be useful to obtain crime scene photos, sketches, or other pertinent documentation from the submitting agency to assist in understanding where the submitted items were found. A review of the medical examiner’s report and/or related emergency medical records is recommended, if applicable.

Test fabric separations conducted on the item of evidence should be done as far away as possible from the areas in question and clearly marked as test fabric separations. Sometimes it may be necessary to acquire an item of similar material composition and physical condition in order to conduct the various tests.

Test fabric separations should mimic the questioned evidence fabric separations as closely as possible. For example, if the victim stated the suspect tore her clothing by hand, including a seam, then the scientist will attempt to tear, by hand, an undamaged similar seam in the same article or in a similar article.

Obtain permission from the submitting agency and/or assigned prosecutor to use the submitted damaged evidence item for test separation experiments, prior to performing any such experiments. These experiments will permanently alter the condition of the submitted item(s), so the item(s) are to be fully documented before use in any experiments.

A stereomicroscope with transmitted and incident light and a rolling stereomicroscope, all preferably with magnifications up to 100X, will be utilized during damage assessment analysis. A polarized light microscope and a SEM may also be required for certain portions of the analysis.

29.4.2 NOTE TAKING

The damaged area(s) must be documented. Special focus should be placed on areas where a specific implement was included, or excluded if significant, as making the damage. The documentation must include some sort of imaging, which may include photography and/or scanning with a copier. The documentation must include some method of relating the images of the damage to the location on the item, which may include photography and/or detailed sketches. The documentation must include detailed written descriptions of the damaged areas. Close-up images (with scale) are necessary of the ends/edges of the damaged areas at the separation point(s), of material adhering to these areas, and of material adhering to the implement(s) suspected of causing the damage.

29.4.3 PACKAGING

Follow the guidelines in the Trace Evidence Recovery section.

The nature of the damage and the items submitted will dictate the packaging. A different type of packaging method may be necessary after examination in order to retain the integrity of the damaged area(s).

All significant, inclusionary and exclusionary, test separation experiments are to be made a new item of evidence and returned to the submitted agency. The test experiments are to be packaged in such a way as to preserve the integrity of the damage created.

29.5 INTERPRETATION AND REPORTING

A sufficient number of test separation experiments are necessary to document/support the reproducibility of the damage observed in the questioned item. The characteristics of the questioned damage must be seen in the experiments using the specific questioned substrate, or like substrate, and the questioned implement, if available.

If there are numerous areas of damage and/or different types of damage, it may be possible and/or requested to sequence the damage, address the possible position(s) of the victim and/or suspect, and evaluate the amount of force used. Experiments will need to support any such conclusions relating to these determinations. The notes need to reflect the thought processes, parameters considered/controlled, and the experiment(s) design. The number of variables involved may preclude any definitive conclusion(s).

It may be useful to embed a digital image of unique or specific damage into the laboratory report to assist the reader in understanding the damage and clarify written descriptions of the damage.

The total number and different types of damage observed should be addressed in the report. All damage may not be able to be associated with a particular type of implement or cause. However, if there are different damaged areas that exhibit the same/similar characteristics and are different from the other damaged areas, it is possible these may be grouped together even though an implement was not identified. This may assist in the investigation to indicate that additional weapon(s) need to be located and/or a portion of a suspect/victim’s story does or does not fit.

29.6 QUALITY ASSURANCE

Permanently mark and identify areas used for test separation experiments on the submitted evidence items.

29.7 SAFETY

Take appropriate safety precautions when creating and/or simulating damage.

30 FIBER AND TEXTILES

30.1 INTRODUCTION

Fiber and textile examinations are performed to characterize, classify, and compare fibrous evidence that may help to establish an element of a crime or an association between two or more persons or between a person and a place.

Fibers can be composed of natural materials or man-made. Natural fibers can be from products originating from plants, animals, or minerals. Man-made fibers are made of polymers either from regenerated natural products or from synthetic materials.

Textiles are manufactured products that are comprised (at least in part) of fibrous materials. Typical textiles for forensic analysis are fabrics and cordage, but materials such as cloth-backed tapes and some building materials may have a fiber component which can be examined with these same techniques.

The following procedures are a modification of the Forensic Fiber Examination Guidelines (the original, the 2011 updates, and the 2014 updates) from the Scientific Working Group for Materials Analysis (SWGMAT).

30.2 ADVANTAGES AND LIMITATIONS

When fibers are associated with a specific source, such as fabric from the victim, suspect, or scene, a value is placed on that association. The strength of the association is dependent upon many factors including:

- Fiber type or types found
- Fiber color or colors
- Number of fibers found
- Fiber location or locations
- Fabric type or types
- Multiple fiber associations
- Nature of contact
- Fiber transfer and persistence

Whether a fiber is transferred and detected is also dependent on the nature and duration of the contact between the suspect, the victim, or both and the persistence of the fibers after they have been transferred.

30.3 APPARATUS AND EQUIPMENT

In order to perform an identification/comparison, the number of analytical tests performed is left to the discretion of the scientist; however, at a minimum, a fiber examiner must employ a stereomicroscope, a comparison microscope, and a compound light microscope equipped with polarized light capability. Using the comparison microscope an examiner must view questioned and known fibers side by side at the same magnifications in visible light, and alternative lighting, such as polarized light or fluorescent lighting, if the equipment allows. For color comparison a scientist must employ compound comparison microscopy along with one analytical test (e.g., MSP or TLC). FTIR is required for comparisons involving polymeric fibers. Elemental analysis may be required when comparisons involve unusual pigments.

30.4 PROCEDURE

30.4.1 <u>CASE APPROACH</u>

There are four basic activities involved in an analysis: overall case assessment, collection of fibers, preparation of the sample for analysis, and analysis using appropriate methods. Although these activities are independent of each other, anyone can have a significant effect on another. The scientist should perform a combination of methods that have the greatest potential for discrimination between samples.

Fiber comparisons consist of determining if a questioned fiber(s) exhibits the same color, chemical, microscopical, and optical properties as fiber(s) comprising part or all of a known sample. Table 1 lists recommended techniques for analysis and comparison of fibers.

Table 1: Analytical Techniques for Fibrous Materials

Physical Characterization	**Optical Characterization**	**Chemical Analysis**	**Color/Dye Analysis**	**Instrumental Analysis**
Stereomicroscopy	PLM	Solubility	Comparison microscopy	FTIR
Light microscopy /comparison microscopy	Light microscopy /comparison microscopy	Staining (natural fibers)	MSP	SEM-EDX/XRF
SEM	Fluorescence			
Physical test (dry twist, ashing, etc.)			Raman	Raman

Shaded boxes are highly recommended or required.

30.4.2 ANALYSIS

Preliminary Examination
Fibers should be first examined with a stereomicroscope. Physical features such as crimp, length, color, diameter, luster, apparent cross section, damage, and adhering debris should be noted. Fibers may then be tentatively classified into broad groups such as synthetic, natural, or inorganic. If the sample contains yarns, threads, or sections of fabric, their construction should be recorded (See Appendix C: Fabric and Cordage Terminology).

Photography of the item prior to conducting any analyses is recommended to provide documentation of its original condition. Any physical damage (e.g., worn, cut, broken, frayed) should also be documented at this time. Prior to textile analysis, other evidence (e.g., hair, blood, paint) that may require additional examination should be documented and collected.

A questioned material (e.g., a piece of fabric, yarn, tuft of fibers) must not be brought in contact with the known fabric from which it is suspected to have originated until a preliminary examination of the questioned specimen has been performed.

The condition of a questioned material (e.g., shape, position, layers or relation of one yarn to another) should not be altered before a preliminary examination for damage has been conducted.

A sample to be used for composition testing should not be cut from ends of yarn or edges of fabric if there is a possibility of physically matching a questioned item to a known item. It is recommended that the known sample be collected away from the existing edge(s) and the location marked.

Sample Handling
Items of evidence may be visually inspected and forceps used to remove fibers of interest. Simple magnifiers and stereomicroscopes, with a variety of illumination techniques, may also be employed. Other methods such as tape lifting or gentle scraping are usually conducted after a visual examination. Tape lifts should be placed on clear plastic sheets, glass microscope slides, or another uncontaminated substrate that facilitates the search and removal of selected fibers. In order to make viewing and recovery of fibers easier, tapes should not be overloaded. The tape lifts or any material recovered from scraping should be examined with a stereomicroscope and fibers of interest isolated for further analysis.

Fibers on tape lifts may be removed using tweezers, other microscopic tools and solvents. Tape should not be attached to paper or cardboard.

Mounting Media
Fibers that are to be microscopically examined and compared at higher magnifications must be mounted in an appropriate mounting medium. When using a comparison microscope, the same mountant should be used for both questioned and known fibers. Many suitable media are available as temporary and permanent fiber mounts. The choice of mountant (e.g., Cargile Refractive Index Liquids) depends on the particular application(s).

An examiner should be aware of the possible deleterious effects that a mounting medium (especially a solvent-based medium) may have on textile fibers, particularly when mounted for a long time. It is preferable that the mounted fibers previously examined microscopically be used for chemical analysis. If fibers must be removed for further testing, the mounting medium should be removed with a solvent that will not alter the fiber.

If a solvent-based mounting medium is used for refractive index determination, the index of the mountant should be checked periodically against solid refractive index standards and, if necessary, readjusted to its proper value by the addition of solvent. Additionally, the refractive index of the medium can be measured directly and the value recorded by the examiner. If such a medium is used for permanent mounts, the examiner should be aware of the different refractive indices for the fluid medium and the resin after solvent evaporation.

Liquids used for exact refractive index determinations have a known refractive index for n_D with an accuracy to ±0.0005 refractive index units. To make appropriate temperature corrections, values for the temperature coefficient (dn/dt) for each liquid and a thermometer covering the range of 20-30°C, calibrated in tenths of a degree, should be available. High dispersion liquids (V<30) are desirable for dispersion staining and the Becke line method. Cargille refractive index liquids are suitable for this purpose and are recommended for refractive index measurements of fibers.

Optical and Physical Characteristics of Fibers
Color: The color should be observed in transmitted light, with a blue daylight filter or other suitable color correction in the light path if needed. It should be noted whether fibers are dyed or pigmented. Variation in color along the length of individual fibers or between fibers in a sample should also be noted.

Dichroism: Dichroism may be exhibited by certain dyed or pigmented fibers, as well as some mineral fibers. Dichroism is observed by viewing a fiber in plane polarized light, oriented parallel to the direction of the polarizer, then rotating the stage 90 degrees. The substage iris diaphragm should be opened to a low contrast position for this observation. Any change in color should be noted.

Refractive Index: The refractive indices of a fiber may be determined by several methods. Whatever the method used, determination of $n_{\parallel}$ and $n_{\perp}$ should be made by using plane polarized light with the fiber aligned parallel and perpendicular to the privileged direction of the polarizer, respectively. The vibration direction of the polarizer should coincide with the horizontal line of the eyepiece graticule.

Birefringence: For a fiber displaying two refractive indices, birefringence is defined as $n_{\parallel} - n_{\perp}$. When measuring retardation of a fiber using a tilting compensator or quartz wedge, one must assure no error has been introduced due to differences in dispersion of birefringence between the compensator and the fiber. This is of special concern with the examination of fibers with high birefringence. The birefringence of noncircular fibers may be estimated by measuring both retardation and thickness at two pints along the fiber that represent their highest and lowest values.

Sign of Elongation: For a birefringent fiber, the sign of elongation is positive (+) if $n_{\parallel} > n_{\perp}$ and negative if $n_{\parallel} < n_{\perp}$. All common manufactured fibers with a birefringence higher than 0.010 have a positive sign of elongation. Full or quarter wave compensators are commonly used to make this determination for fibers with birefringence less than 0.010, which exhibit first order gray or white retardation colors. To determine sign of elongation for a low birefringence fiber, the fiber is oriented perpendicular to the orientation of the compensator between the crossed polars. A full wave (first order red) compensator is inserted with the slow direction (Z direction on the compensator) parallel to the length of the fiber. Colorless fibers with a positive sign of elongation will appear blue in this orientation; white fibers with a negative sign of elongation will appear orange.

Diameter: The diameter of circular fibers can be measured using an eyepiece graticule/reticule or an image analysis system calibrated with a micrometer slide for each microscope objective or magnification. Noncircular fibers require special considerations. If fiber diameters are not uniform within a sample, or if different aspects are presented by non-circular fibers, a determination of the range of diameters exhibited by the sample is recommended. Measurements should be made at the highest magnification that is practical, with the substage iris opened to a position of low to moderate contrast, so that the edges of the fiber are defined but not too dark.

Cross-Section: When viewed longitudinally on glass slides in a suitable mountant, the apparent cross-sectional shape of fibers can often be determined by slowly focusing through the fiber (optical sectioning). Actual fiber cross-sections provide the best information on cross-sectional shape. Physical cross-sections from fibers as short as 1 mm can be prepared. Manufactured and vegetable fibers may be sectioned anywhere along their length. Animal hairs may be sectioned to yield additional identifying characteristics. When observing manufactured fiber cross-sections, the general shape, distribution of delustrant, and/or pigment particles; the presence and size of spherulites or voids; depth of dye penetration; and surface treatments should be recorded when present. The fiber dimensions measured from a cross-section can be used for the calculation of birefringence and the determination of the modification ratio.

Modification Ratio: The modification ratio of non-circular fibers can be calculated by obtaining an image of the fiber cross-section, and using a circle template or image analysis system to determine the sizes of the circumscribing and inscribing circles for that shape. The modification ratio is the ratio of the larger circle's diameter to the small circler's diameter. This value may help to identify a particular manufacturer or end use of a fiber.

Delustrant: The presence of absence of delustrant particles, as well as their size, shape, distribution, abundance and general appearance, are useful comparative features. Also, the presence of delustrant shows conclusively that a fiber is manufactured, rather than natural. Delustrant particles, while not indicative of any particular generic fiber type, can be characteristic of end use properties needed by a manufacturer.

Surface Characteristics: Fiber surface characteristics such as manufacturing striations, damage, coatings and surface debris (e.g., blood, or other foreign material) should be recorded. Surface striations are more apparent in a mounting medium of refractive index significantly different from those of the fiber.

Fluorescence: Fluorescence may arise from the fibers themselves, dyes, other additives from the finishing process, laundering, chemical treatment/damage, as well as from surface debris. Fibers should be mounted in a low- to non-fluorescent medium to observe fluorescence. Examination using various combinations of excitation and barrier filters is desirable. At each excitation wavelength, the color and intensity or absence of fluorescence emission should be noted.

Additional Physical Characterization Techniques

Scanning Electron Microscopy: Applications of SEM-EDS to fiber analysis include the characterization of fiber cross-sections, identification of pigments, delustrants and nanoparticles by elemental analysis, fiber damage due to cuts and tears, trace debris on fibers, surface feature modifications such as washer/dryer abrasion, and acid washed treatment of denim garments. Surface imaging using SEM as an aid in the identification of animal hair scale structure has been reported.

Chemical Analysis
Solubility: Solubility testing can provide supplemental information to optical methods of characterization, but since it is a destructive method, it should be used only when sufficient sample is available and non-destructive methods have been exhausted. Possible reactions of fibers to solvents include partial and complete solubility, swelling, shrinking, gelling, and color change. Appropriate reagent controls will be employed. It is desirable to view known and questioned fibers simultaneously under a microscope when comparing their solubilities.

Color/Dye Analysis
Comparison Microscopy: For proper comparison of fiber samples and for the preparation of test slides, both specimens should ideally be mounted in the same medium, using slides and cover slips of the same type and preferably from the same package. In cases where comparisons are being made to slides prepared by another examiner and these parameters cannot be duplicated, an examiner should use caution in making an exclusion based on minor differences in the appearance of two fiber samples, particularly minor differences in apparent color. In such cases, extraction and remounting of fibers may be necessary in order to reach a firm conclusion.

Ultraviolet-Visible Spectroscopy: Occasionally, an aromatic solvent reduced mounting media, such as XAM, can have an adverse effect on some fiber dyes and fluorescent brighteners, dissolving them and allowing them to diffuse from the fiber. This normally happens very quickly after mounting. If on mounting the known sample, “bleeding” is apparent, another mountant should be used for the preparation of the known and questioned fibers. It is important that a minimum amount of mountant be used consistent with a thin, flat void free preparation. Ensure that the long axis of the fiber remains parallel (as far as possible) to the plane of the microscope slide surface.

Known fiber sample selection should represent the complete range of fiber colors and dyeing depths represented in the known fabric, yarn, or other fiber source. Care should be taken to ensure that the sample reflects the extent of wear, biological deterioration, thermal, and/or mechanical change, bleaching, and laundering artifacts exhibited by the item. Known fibers should be well separated (microscopically) and mounted in the same manner as the questioned fibers, ensuring that the fibers are mounted in a single layer.

In systems with rectangular apertures, it is recommended to orient the fibers in the same direction N/S or E/W as the instrument response may vary with orientation. Circular or square apertures are not as sensitive to sample orientation; however, all samples should be oriented in the same direction for comparison purposes.

Single fibers may not be uniformly dyed. Natural fibers generally exhibit non-uniform cross sections along their length. These conditions can produce variations in color depth at different places along a fiber. Measuring sites should be chosen to avoid obvious inhomogeneities occurring within the area being measured. Multiple locations along a single fiber or fibers may need to be scanned. More scans may be needed if it is necessary to produce a representative mean absorbance curve and standard deviation curves for an individual fiber.

Synthetic fibers may yield good results with fewer scan locations than natural fibers. Known samples of synthetic fibers may exhibit dye variations among the fibers. These sets of fibers should be sampled to exhibit the widest visual range of dyeing depths in each of them.

When analyzing known samples, it is recommended that at least five fibers from a synthetic fiber sample or ten fibers from a natural fiber sample should be selected. Both the extremes and midranges of apparent dyeing depths should be represented in the scans. Take care to sample a variety of fiber thicknesses and cross sections.

When evaluating usefulness of UV-VIS over visible spectroscopy, several factors may need to be considered. If the fibers are mounted on glass slides, they will need to be removed and mounted on quartz slides with quartz cover slips for UV-VIS spectroscopy. Some fiber polymers (e.g., polyester) absorb moderately to strongly in the UV range, such that analysis in this range may be difficult or pointless. For other types of fibers, valuable discriminating information may be obtained in the UV range.

Spectra may be recorded in transmittance or absorbance according to operator preference. It is recommended, however, to use absorbance when recording spectra from very dark fibers. Averaging and spectral derivatives are also an option.

Questioned and known spectra can be compared by overlapping them or by plotting them sequentially on the same graph. Each questioned fiber spectrum must be compared to the known fiber spectra, to determine if a positive association is found. The position of the peak maxima (nm), peak width, and peak intensity must all be considered.

Instrumental Analysis

Infrared Analysis:
Sample preparation should be similar for all fibers being compared. Except when using ATR, fibers should be flattened prior to analysis in order to obtain the best quality spectra. Flattening the fiber alters the morphology, and therefore, the minimum length of fiber necessary for the analysis should be used. Flattening the fibers can alter the crystalline/amorphous structure of the fiber and result in minor differences in peak frequencies and intensities. This must be taken into consideration when making spectral comparisons. Leaving fibers unflattened, while allowing crystallinity-sensitive bands to be observed unaltered, results in distortion of peak heights due to variable pathlengths. In certain situations, a combination of both approaches may be advisable. Fibers analyzed via ATR do not require flattening prior to analysis as the fibers are flattened by a hand-operated press when the sample is mounted against the diamond surface.

The flattened fiber may be mounted across an aperture, on an IR window, or between IR windows. It is important that the longitudinal plane (flattened surface) of the fiber be as nearly parallel to the IR window or other mount as possible.

Where several fibers are mounted on or in a single mount, they should be well separated (physically) so that their positions can be unambiguously documented for later retrieval, reanalysis, or both, and to prevent spectral contamination from stray light that might pass through another fiber.

Infrared spectrometers and microscopes exhibit polarization bias. It is essential that fiber alignment be consistent throughout an analysis.

Samples should be focused as close to the center of the sample volume as possible and centered on the optical axis of the system. The condenser should be focused and re-centered if necessary. This is best accomplished using a circular field aperture.

The detector measurement aperture width should be adjusted to just slightly less than the width of the fiber. The aperture length may vary with sample geometry but should not be so great as to allow the detector to be saturated when acquiring a background spectrum.

Identification of Manufactured Fibers
After preliminary examination and general classification by use of a stereomicroscope, the generic fiber type can usually be identified using a polarized light microscope. Synthetic fiber types are best identified

by determining optical properties such as refractive indices, birefringence and sign of elongation. FTIR is recommended to identify sub-groups within synthetic fiber types. Elemental analysis by SEM-EDS is useful in sub-typing glass fibers, as is refractive index measurement. Physical features such as diameter, cross-section, modification ratio and surface treatment, while not necessarily characteristic of a particular fiber type, may aid in identifying or eliminating possible end uses and are also important comparative features. Features such as color, dichroism, delustering and fluorescence are primarily of use for comparison of different fiber samples.

Glass fibers are often encountered in building materials, insulation products and in fabrics. Glass fibers are also called manmade vitreous fibers. Based on the starting materials used to produce glass fibers, they can be placed into three categories: fiberglass (continuous and noncontinuous), mineral wool (rock wool and slag wool), and refractory ceramic fibers (glass ceramic fibers). Single crystal and polycrystalline refractory fibers such as aluminum oxide, silicon oxide, silicon carbide, zirconium oxide, and carbon are not included because they are not considered glass fibers.

Glass fibers are normally identified by their morphology and isotropic nature. The presence or absence of coating resins and of spheres and slugs may indicate an end use and are also useful comparative features. Light microscopy together with classical immersion methods may be used to determine the refractive index for the classification and comparison of glass fibers. The dispersion staining technique may be used when determining the refractive index and variation of the refractive index within a sample. SEM-EDS may be used to provide elemental composition for the purposes of classification and comparison.

Identification of Natural Fibers
Most natural fiber types are best identified by their physical and morphological characteristics. Optical properties such as refractive indices and birefringence are of more limited use in identifying or comparing natural fibers than in the analysis of manufactured fibers, with the exception of identifying specific types of asbestos. For natural fiber comparisons, color is the main discriminating characteristic and microscopical color comparison should be supplemented by techniques such as MSP.

Identification of Animal Fibers: The principal morphological features of animal hairs are the root, medulla, cortex, and cuticle; shield size and subshield structures are also useful traits for species identification. Medullary and cortical structures are best observed on hairs mounted on a slide with suitable mounting medium. Cuticular scales are best observed on replicas cast in a transparent polymer (scale casts). Scale counts (scales per 100 micrometers) can help distinguish specialty fur fibers. Silk, a protein fiber produced by caterpillars, has morphological features that differ from other animal hairs. Some features of silk include cross-over marks, and a wedge to triangular cross section with rounded corners. In textiles, silk may occasionally be seen as paired fibers cemented together, but is most often found as single fibers.

Identification of Vegetable Fibers: Plant fibers may be encountered as technical fibers in cordage, sacks, mats, etc., or as individual cells (ultimates) in fabrics and paper. The examination of technical fibers should include an observation of a cross section. Additionally, a chemical test for lignin may be done. Technical fibers should be macerated, fabrics teased apart, and paper re-pulped for the examination of the shape of the fiber ultimates. The presence, type, and distribution of dislocations in the fibers should be noted. The direction of twist of the cellulose in the cell wall can also be determined. Other characteristic cells should be noted and compared to authentic specimens.

Wood pulp fibers from paper or cardboard can be distinguished from other cellulosic fibers by morphology or staining tests. Distinction between hardwood and softwood and more specific genus or species may also be possible for wood fibers.

Identification of Inorganic Fibers: Natural mineral fibers are commonly called asbestos, which is a general term for many naturally occurring fibrous hydrated silicate minerals. The asbestos minerals include chrysotile, amosite, crocidolite, fibrous tremolite/actinolite, and fibrous anthophyllite. Chrysotile belongs to the serpentine group of minerals that are layer silicates. The other asbestos minerals are amphiboles and are classified as chain silicates. Asbestos fibers alone or mixed with other components may occur in building materials and insulation products. Chrysotile is the only asbestos mineral that would be encountered as a woven fabric, but any of the asbestos minerals may be found in pressed sheets such as gaskets. Take care when analyzing asbestos fibers since they are considered a potential health hazard.

Asbestos minerals can be easily identified by their optical properties using polarized light microscopy. Although not considered essential, the dispersion staining technique is extremely helpful. SEM-EDS can also be used to characterize the asbestos minerals. Nonmicroscopical techniques for asbestos identification include x-ray diffraction and infrared spectroscopy.

Microscopical Fiber Comparison
If both the known and questioned samples are available, fibers are compared to determine whether or not they share similar physical characteristics and could have originated from a common source. Samples from different incidents or items may be compared to one another to determine if there are fibers that may share a common origin.

Fibers may first be examined with a stereomicroscope to select fibers that warrant further comparison. For a detailed comparison of color, cross-section, delustrant and overall microscopical appearance, a comparison microscope must be used. The side-by-side, point-by-point examination made possible by a comparison microscope is the best technique to microscopically compare fibers. Examination of specimens on two separate slides in rapid succession on a single compound microscope is not an acceptable method of comparison, as visual color memory in such a case is not sufficiently reliable for fibers of similar, but slightly different shades.

Characteristics such as birefringence, modification ratio and diameter may be measured separately on different fiber samples and compared on a comparison microscope to the extent possible. Photography may be utilized to capture features of the fiber comparison for later detail.

Fabric and Cordage
Prior to any analysis of the fibers comprising a fabric or cordage, the fabric or cordage should be examined for physical matches, pattern evidence, damage such as thermoplastic fusions, and cut/tear marks, etc. Any adhesives or other material used in bonding fabrics, carpet backings, etc., should also be noted.

Physical Match: Physical matches should be considered if two pieces of fabric or cordage having cut or torn ends are to be compared. A physical match must be documented by photography, sketching or through a thorough description of the condition of corresponding threads and their relative positions in the damaged areas on the questioned and known pieces (“longs and shorts”).

If a physical match is not possible, comparison of the color, pattern, construction and composition of the items in question should be undertaken to determine if they are similar and if the items could have originated from the same source.

Fabric: A fabric examination is primarily a process of deconstructing the fabric by dissecting its constituent elements. Each of these elements can have a number of sub-elements, all of which must be characterized to complete the examination for comparison purposes. These elements include, but are not limited to, the following:

- Overall
    - Construction (woven, knit, nonwoven)
    - Yarn counts in warp and weft direction
    - Color(s) and design

  - Type of dyeing or printing
  - Sewing threads, buttons, decorations, etc.
- Yarns
  - Staple or filament fibers in yarns
  - Diameter
  - Yarn twist
  - Number of plies
  - Direction of twist of each ply
  - Number of filaments in each ply, if feasible
  - Composition of yarn
  - Blend of two or more types of fibers within each ply

Cordage: A cordage examination is primarily a process of deconstructing the rope or cordage by dissecting its constituent elements. Each of these elements can have a number of sub-elements, all of which must be characterized to complete the examination for comparison purposes. These elements include, but are not limited to, the following:

- Overall
  - Diameter
  - Braided or plied
  - Direction of twist
  - Number of crowns or turns per inch
  - Number of plies/strands/braids
  - Core, if any
  - Color(s)
  - Coatings, if any
  - Tracers, if any
- Plies/Strands/Braids
  - Staple or filament fibers
  - Twisted or non-twisted
  - Direction of twist for each
  - Crowns or turns per inch for each
  - Number of filaments in each, if feasible

After the construction has been established, the constituent fibers should be analyzed with the appropriate microscopical and instrumental techniques. Additional characteristics may be used if necessary to adequately describe the cordage.

Fabric Impressions: An impression made from an item of clothing, for example, in a vehicular accident or other situation, may provide evidence. The weave or knit pattern of a fabric impressed onto another surface or into blood, grease, etc. can be compared to the garment in question. Fabric impressions of a known fabric can be made with impression material used for footwear impressions and then transferred onto a piece of paper or digitally reproduced onto a clear sheet of plastic to use as an overlay for direct comparison with the evidence impression. Portions of a garment that were stitched or have other identifying features may help align the patterns. The count of the warp and weft yarns per inch and any other identifying construction indicators must be comparable between the impression and the fabric. Fibers left in the impression should be recovered and examined further as they may add additional significance to the overall fabric and fiber examination.

Thermoplastic Fusions: Fiber fusions may occur between textiles and various plastic or polymer coated surfaces due to the heat caused by the friction of impact, such as from high-speed impacts with a vehicle. Partially fused fibers may be found in an impression on a vehicle hood, interior of a car, etc. Photographs with a scale should be taken prior to removing any fused fibers to preserve the impression. Care must be taken not to damage the impression when attempting to remove fibers partially fused to the surface. The removed fibers can then be compared to a known sample and, if necessary, the thermoplastic properties can be assessed (e.g. melting point).

Cut/Tear Fabric Damage: Examination of the cuts and tears in fabrics can offer information as to the implement that may have produced the damage. Analysis and documentation of the shape, pattern, and dimensions of the damage is followed by analysis of the edges of the cut/torn yarns. The analysis should be both visual and with the aid of magnification, either with a microscope or magnifying lens, to determine if the individual yarns of the fabric are cut or torn. A scanning electron microscope may add further detail to the examination of the cuts and tears of the individual fibers.

Test cuts may be made with questioned implements to see if they make cuts or tears that are consistent with the shape, pattern and dimensions of those found on the evidence. These test cuts should be made after all other forensic examinations, such as DNA and latent fingerprint analyses, have been conducted on the item. Test cuts are made in an undamaged portion of the item corresponding to where the damage originally was noted or, if it is too damaged, in a similar type of fabric or garment. The fabric should be placed on an appropriate substrate prior to producing the test cuts/tears. Examples of suitable substrates include cardboard boxes of sufficient strength to withstand the insertion of a blade or other cutting implement, Styrofoam, gel blocks and body replicas. In addition to comparing the test cuts/tears to those found in the evidence, the orientation of a knife in relation to the cut mark may be determined. This can be done if a single-edged blade was used, by finding the characteristic “V” shaped notch at one end of a cut/tear mark, which corresponds to where the flat portion of the blade entered the fabric. It should be noted that the dimension of the test cut/tear may not correlate on a one-to-one basis with the knife due to fabric stretch.

30.4.3 NOTE TAKING

The examiner’s notes should reflect the particular characteristics observed in the microscopic examination, any calculated values, descriptions, diagrams, or photographs. The examination notes must contain sufficient detail to support the conclusions such that another qualified examiner could reach the same conclusion based on the notes or documentation.

30.4.4 PACKAGING

Fibers can be packaged in any type of sealed envelope, bag or vial. Use containers that are small enough so that the fibers could be easily recovered and reexamined at a later date. Evidence packaging materials should have all seams and openings sealed to eliminate the possibility of lost fibers.

30.5 INTERPRETATION AND REPORTING

Fiber identification consists of determining the generic class of a fiber, which generally follows the Federal Trade Commission (FTC) Guidelines. This analysis requires a sufficient number of examinations to unequivocally place the fiber in question into one and only one generic class (see Appendix B: FTC Generic Names and Definitions for Manufactured Fibers).

In the interpretation of MSP data, a spectral inclusion is when the questioned spectrum falls within the range of the known spectra when considering the curve shape and absorbance values. A spectral exclusion is when the questioned spectrum falls outside the range of the known spectra in either curve shape or absorbance value. An inconclusive result is when there are no significant points of comparison in either the questioned or the known spectra (e.g., spectra from microscopically black or from very pale fibers, which are outside the dynamic range of the instrument).

Successful identification of fiber polymers by IR spectra depends on the experience and familiarity with fiber reference spectra. Spectral identification is accomplished by comparison with spectra of known reference standards. A library of reference IR spectra is essential. A library of reference spectra obtained using the same technique used for the unknown fiber is also desirable.

If the statement “similar in all microscopic characteristics” is used in the report then $n_{\parallel}$ and $n_{\perp}$ for the fibers in question must be determined. If you compare all the fibers using, for example, Permount® then the comparison statement must read “similar in all microscopic characteristics relative to Permount®” (or the name of any other media that you have used). The reason for the precise wording is that many different fibers have similar refractive properties in Permount® and as a result, the analytical data is not as specific as identifying $n_{\parallel}$ and $n_{\perp}$ by the bracketing method.

If a physical match is found, it should be reported in a manner that will indicate that the two or more pieces of material were at one time a continuous piece of fabric or cordage. If no physical match is possible/found, a complete fabric/cordage comparison, including construction and composition can be performed. If, during this examination, the items are found to be the same in all tested characteristics, then the examiner reports that the two objects exhibit the same color, construction, and composition and are consistent with originating from the same source.

Fabric impressions associated to a known sample should be reported as consistent in all points of comparison between the questioned and known impressions; however, the results should not be limited to that specific garment as having produced the impression due to the bulk manufactured nature of textile material. It is rare that a positive identification can be made for fabric impression evidence and care should be taken to account for possible fabric stretch or distortion. Fabric impressions without fabric transfer associations generally do not rise to the same level of significance as those with a transfer.

In fabric damage cases, when comparing test cuts/tears to documented damage, and an association is found, the report wording should be limited to a statement that the cut/tear marks are consistent in size, shape and general appearance with having been made by that weapon or another implement of similar characteristics and dimensions. If a transfer of fibers has been found on the weapon, the significance of the conclusion in the report may be strengthened. However, the report wording concerning the fabric damage should still be limited to the cut/tear analysis wording without overstating the results or precluding other possible weapons of similar nature from having produced the damage.

30.6 QUALITY ASSURANCE

Care must be taken to avoid cross-contamination of samples. This can be accomplished by examining questioned and known items in separate areas and/or at different times. The work area and tools must be thoroughly cleaned and inspected before examining items that are to be compared.

31 FOOD, GASTRIC CONTENTS, AND VOMIT

31.1 INTRODUCTION

The examination of gastric contents, vomit stains and food have probative value in criminal investigations. Digestive processes in the body can take hours to days. Food is broken down and reduced to a liquid mass from which nutrients are extracted by the body. Foreign materials, poisons, toxins, and drugs may be present in the digestive system.

31.2 ADVANTAGES AND LIMITATIONS

The identification of vomit stains may prove or disprove a suspect or victim alibi, support witness testimony, and help in crime scene reconstructions. Vomit traces may reveal food particles that identify a specific food or meal leading investigators to a restaurant in which the victim may have last dined. An unusual food ingredient or an abundance of one type of food may suggest traits or food habits helpful to an investigation. Vomit may transfer from a victim to a suspect environment, e.g., clothing, dwelling or automobile. When stomach contents from a victim and foreign samples from a suspect environment are characterized and compared, similarities in food ingredients may suggest a common origin linking the suspect to the victim.

31.3 APPARATUS AND EQUIPMENT

In addition to basic laboratory supplies and instruments, the lab should have available dichotomous plant cell keys, commercially prepared microscope slide sets and individually prepared food samples including various starches prior to undertaking the identification of food particles, vomit stains and gastric contents. A known fresh human vomit sample is required in enzymatic testing of questioned vomit stains. Other materials needed for vomit testing include whole cow's milk, warming oven, humidity chamber, spot plates, and pipettes. A broad spectrum ultra-violet lamp is useful in searching for vomit stains

31.4 PROCEDURE

31.4.1 CASE APPROACH

In the analysis of food traces a simple identification is all that is required. In the submission of a suspected vomit stain, an enzymatic test may only be needed. In some cases the enzymatic test and the identification of food products in the vomit is performed. Consider contacting the medical examiner who performed the autopsy to determine if a preservative was added to the gastric contents.

31.4.2 ANALYSIS

Food traces
Visual inspection of items of evidence may reveal food traces. Food is commonly encountered as particles and/or smears which can be picked from a substrate with ~~a~~ forceps. When suspected food traces are observed, document in your notes and photograph. Food can also be brushed or scraped from materials over fresh examination paper. The particles are first examined using stereo-binocular microscopy. At this stage many particles can be sorted by shape, color and consistency into a variety of classes such as botanical (e.g., leafy plant), meat (e.g., skeletal muscle), and seeds. Particles are then mounted onto separate labeled microscope slides in a mounting media. PLM is used to characterize and identify food traces. Staining methods can be employed to identify the cytoplasm and cell wall structure. Instrumental methods such as SEM-EDS, XRF and FT-IR may be used to aid in characterization and identification.

Gastric enzyme test
A presumptive screening test for the presence of gastric fluid can be used to identify stains which are suspected to be vomit. A small portion of the suspected stain is placed into a dark porcelain spot plate

along with a known dried vomit sample. Several drops of whole cow's milk are pipetted into each well. An additional well is used to pipette a known sample of just milk. The spot plate is placed into a humidity chamber at 38.0° C for approximately 30 minutes. The spot plate wells are then examined visually and with a stereo-binocular microscope. The occurrence of coagulation or curdling indicates gastric enzymes are present in the sample. The known vomit sample should also exhibit coagulation. The reactions on the spot plate are then documented photographically. Known vomit samples as small as 0.5 mm in diameter have tested positive using this procedure.

Vomit stains
Vomit stains are usually identified by the presence of masticated, partially digested food ingredients in conjunction with a gastric enzyme test. Items are examined for the presence of vomit stains visually and with the use of a stereomicroscope. Vomit stains may fluoresce using a broad spectrum ultra-violet light which can be used. Be aware that long periods of exposure of materials with UV light may degrade or destroy DNA. Therefore consult with the DNA section prior to its use. The stain is measured, photographed and documented in notes.

Particles from dried vomit stains can be removed with ~~a~~ forceps or scraped off with a razor blade and re-suspended in distilled water on microscope slides.

A portion of the sample can be picked off and placed in a small petri dish and suspended in distilled water. Materials are thus quickly cleaned and separated by density. The particles floating on the surface can be quickly mounted for characterization by light microscopy for identification. The remainder can be either dried and particle picked to a slide or placed in another solvent for cleaning and mounting.

Staining methods can be used to assist in identification of cell walls and cytoplasm (e.g., iodine stain for starch).

Instrumental methods such as SEM-EDS, XRF and FT-IR may be used to aid in characterization and identification of food particles.

Gastric contents
Record date of collection, added preservative if any, and storage temperature, photograph contents, weigh contents, and describe color and consistency. Gastric contents should be stored refrigerated or frozen. Contact the medical examiner's office to determine if a preservative was added to the gastric contents. If none was added consider added approximately 5 milliliters of a preservative such as formalin to the entire portion to be examined to prevent bacterial growth.

Examine contents in a petri dish or equivalent container visually and using a stereo-binocular microscope looking for any suspected foreign objects such as pharmaceuticals. Consult with other analysts/drug chemists if needed.

Separate out all macroscopic particles (e.g., peas, corn, noodles), characterize and identify if possible. Sieving may be necessary when screening and segregating particles found in gastric contents. Record the screen(s) size used and the particle types recovered from each screen. Consider wet aqueous sieving if gastric contents are thick. Centrifugation may be necessary when separating particles types. If so, follow the directions in use for the centrifuge.

Prepare microscope slides using distilled water or other suitable mounting media and examine, characterize and identify by PLM.

Consider staining methods to assist in identification (e.g., iodine stain for starch).

Instrumental methods such as SEM-EDS, XRF and FT-IR may be used to aid in characterization and identification.

31.4.3 NOTE TAKING

Standard note taking practices should be employed.

31.4.4 PACKAGING

Package glass containers carefully to prevent breakage. Gastric contents samples should be returned to the user agency in a refrigerated or frozen state.

31.5 INTERPRETATION AND REPORTING

The level of particle identification is dependent upon case specific needs and the knowledge and training of the analyst. Meat, regarded as the flesh of mammals and birds, can be identified as skeletal muscle with the observation of striations and dark stained nuclei using aqueous toluidine blue, but not to a specific group (e.g., pig, cattle, bird).

Gastric contents can be examined to determine a subject's last meal in an investigative inquiry. The finding of different food ingredients may assist in the identification a general type of cuisine. For example the finding of bok choy, shrimp and rice may suggest an Asian recipe. Be aware of over-interpretation in suggesting specific restaurants or locations. Food particles can be identified at a variety of levels dependent upon case needs and analyst training (e.g., 'starch' corn starch, wheat starch). A botanical genus species name for a fruit or vegetable is often not performed or required.

The identification of vomit relies upon the presence of a positive gastric enzyme test with or without masticated food products. If a stain contains no identifiable food products but tests positive for gastric enzymes (presence of curds), it is considered a positive test for the presence of vomit. If a stain tests weakly positive for gastric enzymes (only a trace of curds) and contains masticated food products, the stain is considered as possible vomit. If a stain tests weakly positive for gastric enzymes and does not contain masticated food products, the stain is considered inconclusive for the presence of vomit. The enzymatic activity within vomit stains reportedly decreases with time, therefore if a stain tests negative for vomit and exhibits masticated food products it is considered inconclusive.

31.6 QUALITY ASSURANCE

Positive and negative controls will be performed and documented in case notes for the gastric enzyme test. The scientist should take care to employ proper methods of cleaning tools, laboratory equipment, and work areas between samples in order to prevent contamination.

31.7 SAFETY

Gastric contents are a biohazard and appropriate precautions should be taken when handling samples.

32 GENERAL CHEMICAL ANALYSIS

32.1 INTRODUCTION

A wide variety of substances may be of interest in law enforcement situations/criminal cases and thus could be submitted to the Crime Laboratory for analysis and identification. Items submitted can be a myriad of organic or inorganic materials. Requested submissions can include analysis for target analytes, comparisons to determine if a sample has been adulterated, or unknown substances.

Analysis for a target analyte is examining a product for the presence of a known contaminant. This contaminant can be a single compound, such as identifying bank dye, pepper spray (OC spray), heavy metals, or rat poison, or for a specific product added to another substance, such as a specific brand of cleaner or perfume.

Comparisons generally require determining if a known material has been adulterated. Submissions will typically include an item, often a food or beverage, suspected to have been tampered with. When at all possible, a sample should be obtained of a known, untainted version of the adulterated material. This comparison sample may be a purchased product or a separate portion of the material from which the suspect item was taken (e.g., coffee from the victim's cup may be submitted along with coffee from the untainted pot).

For unknown substances, the submitting agency may have little or no information about the nature of the substances, which will require a careful and broad based approach when handling the evidence. Because of the unknown nature involved in these types of cases, it may be prudent to obtain the case scenario and/or do research before analysis begins.

Due to the highly variable nature of the items submitted, careful planning is required for both safety concerns and the analytical approach to be taken before work commences. The review of the laboratory request may require thorough coordination between the laboratory and submitting agency to obtain all the information available concerning the case and the items submitted that might have an impact on the handling, storage, and analysis of the item, with documentation of all discussions leading to acceptance or rejection of the item as required in the Quality Operations Manual.

32.2 ADVANTAGES & LIMITATIONS

Acceptance of these types of cases will be predicated upon the evaluation of limitations and whether or not the limitations make it possible to conduct any analysis. Reevaluation of these limitations should occur even after casework has begun.

Analysts should ensure that the proposed methods for the identification of a general unknown have been sufficiently documented and tested to ensure their validity before proceeding with further case analysis. It is often useful to consult literature, manufacturer information, and/or other analysts before beginning the analysis of unfamiliar materials.

In some cases limited sample size may restrict the types of analyses that can be used such that non-destructive methods are preferred.

Limitations due to sample matrix interference such as irreversible absorption/adsorption of the sample into the matrix and co-extraction of matrix materials with the sample, can affect the ability to properly identify an unknown material.

Spoilage of food and other biological materials, if they have not been suitably packaged and stored, or too much time has elapsed since evidence was collected, may make analysis impossible.

Volatile materials, such as pepper sprays and solvents, if not packaged in vapor-tight containers, may evaporate before they can be collected or analyzed.

Appropriate reference samples should be obtained, e.g., gauze, water or other material used to collect the sample or soil from near where the sample was collected. Lack of an appropriate reference sample may lead to a qualified conclusion or the inability to analyze the sample.

With techniques currently available in the WSP Crime Laboratory, it may not be possible to uniquely identify some materials, such as proteins and other biological materials. Some substances may be of forensic significance at levels below the limits of detection of available techniques, such as radiological poisons and active ingredients in rat poisons. In some cases, it may not be possible to identify specific compounds, but still be possible to compare analytical results and determine whether two samples have the same or similar analytical profiles.

32.3 APPARATUS & EQUIPMENT

The evidence submitted will dictate the tools, equipment and instrumentation needed. A wide variety of laboratory supplies, reagents and instruments may be necessary for the analysis of unknown materials. Any and all instrumentation that has been verified may be used. The limitations of the tests performed must be understood by the scientist.

32.4 PROCEDURE

Any identification made must be based on a technique, or combination of techniques, that are of sufficient discriminating power to allow for the identification of the chemical or substance in question. It is critical for the analyst to understand the analytical limitations of the methods selected for analysis, as not every combination of techniques will be sufficient for the identification of every compound. The combination used must reasonably preclude any other similar substances.

Appropriate technical procedures and methods that have been scientifically validated and accepted for use in the field of forensic science will be used. This includes methods and procedures for the sampling, handling, transport, storage and preparation of evidence items, the operation of all relevant equipment and an estimate of the measurement uncertainty where appropriate. All methods and procedures will be documented and readily available for review. Any deviation from a standard technical procedure or method will require that the details of the modification as well as the justification and the authorization be documented in the case notes and maintained as a permanent part of the case file. Even though the instrumentation used in these examinations may have been validated, it is important that their use in a new context be suitable for the analysis in question. Appropriate positive and negative controls will be used to perform testing on unknown materials.

Consideration of the quality and quantity of the sample, the requested analysis, and the case circumstances will determine the tests to perform. Materials may be pure substances or mixtures, and most will require a variety of analytical techniques in order to be characterized or identified. These techniques may include a combination of chemical, physical, microscopic, and/or instrumental testing. Results obtained from early testing will determine the ultimate course of the analysis and the choice of techniques to be used.

The analysis will generally begin with documentation of the appearance and condition of the evidence, noting physical characteristics such as physical state, homogeneity, morphology, texture, color, etc. If necessary, a determination of the weight and/or volume should also be made.

Care should be taken to use chemical, physical, microscopic and instrumental analysis methods appropriate to the sample. Miscibility testing and pH should be considered for their usefulness in the analysis of liquids. Microchemical and instrumental (e.g., CE, XRF, IR) tests can be used to detect the presence of certain ions. Appropriate extraction methods should be used for the analysis of mixtures as necessary. If sufficient sample is available, it may be useful to evaporate the liquid and analyze the residue as a solid sample.

Notes taken by the analyst should take special care to thoroughly document the information obtained on the sample, the needs of the submitting agency in the case, and the reasons for the analytical approach(es) used. The notes will also contain sufficient information on any method validation and/or verification required to show that the choice of method was valid for that case.

For some cases it may be useful to analyze the known/comparison sample before proceeding on to the questioned/tampered material. In a target analyte case, this will allow the scientist to first determine the known material's unique characteristics and more easily determine the presence or absence of the target analyte in the questioned case. This can also test whether a proposed analytical scheme is in fact suitable for the target analyte.

Similarly, for a comparison / adulteration case, initial analysis of the known material is desirable to determine if the questioned sample in fact contains extraneous material. Small differences between a known and questioned sample may not indicate an adulteration, but could result from different storage conditions, typical variations within that type of sample, or other reasons. Often it is not possible to exhaustively identify every component of a complex mixture, such as most foods and beverages, nor to make an exhaustive comparison between such samples.

In the absence of a known comparison sample, it may be questionable whether a given component is extraneous or if it is typical of the type of sample. For example, wine may naturally contain small amounts of GBL or similar compounds; therefore detection of these in wine may not indicate tampering. Conversely, a large amount of an insecticide in any food or beverage could be indicative of tampering or adulteration.

Sometimes evidence will be submitted with requests to find residues of unknown substances. Unstained portions of the substrate, if available, should be tested using the same extraction methods used on the stained areas. A search of clothing or other substrates may be aided by the use of various lighting and visualization methods including alternate light sources and stereomicroscopy. Stains may be outlined with a permanent marker with a portion of the stain collected and individually analyzed. A portion of an unstained area of the clothing is similarly collected and analyzed as a comparison.

32.5 **INTERPRETATION AND REPORTING**

The report must clearly state, in sufficient detail, the analytical approaches used, the results of the analysis, and any limitations of the analytical methodology and reported conclusions and interpretations. The Overview section of the report should be used to give the scope and purpose of analysis.

It may be necessary to include a statement in the report indicating the limits of laboratory analysis if no specific material or contaminant was conclusively identified.

> Example: The presence of plant-based or protein-type skin irritants and allergens could not be determined using the analytical methods available to this lab. The presence of highly volatile skin irritants was not determined.
>
> Example: A complete analysis of some materials may not be possible with current methods and technologies available. In some samples, there may be components that cannot be detected or identified. The Washington State Patrol Crime Laboratory does not have the capability to screen for most biological/protein or radiological hazards.

<u>TARGET ANALYTE</u>

A report for a target analyte analysis will clearly state what target analyte was tested for, what methods were used, and whether or not the target analyte was detected. If the target analyte was not detected, but its presence cannot be entirely ruled out, a statement to that effect will be made in the report. In some cases, where there are indications of the target analyte but no conclusive identification, a qualified conclusion including factors that prevented the conclusive identification may be appropriate.

COMPARISON / TAMPERING

A report for a comparative analysis will clearly state the nature of the sample(s), both known and questioned, what methods were used, and whether differences were found between the known and questioned sample. The significance or insignificance of any such differences must be addressed. If no comparison sample was obtained, this must be stated along with the limitations of this to any conclusions made.

UNKNOWN SUBSTANCE

A report for unknown substance analysis will clearly state the scope of analysis performed and what methods were used. If an identification cannot be conclusively made, but is indicated by analysis, a qualified conclusion including the factors preventing conclusive identification may be appropriate. Though identification may not be achieved, useful investigative information should be included in the report. At times it may be possible to develop a list of possible sources or uses of the identified components.

> Example: The residue in the plastic bottle contained aluminum. The water wash of the residue had an acidic pH and contained chloride. Placing hydrochloric acid and aluminum foil in a sealed plastic bottle is a common method of creating an “acid bomb.”

32.6 **QUALITY ASSURANCE**

Verification of methods and techniques using appropriate positive and negative controls may be necessary to perform testing on unknown materials.

Unknown samples should be compared to known samples when known samples are available.

Treat the questioned samples and any control/standard samples in the same manner, as much as possible. When performing extractions, analyze a blank of the extraction liquid and an undisturbed portion of the substrate, if possible.

32.7 **SAFETY**

It is imperative that analysts take appropriate precautions during the analysis of general unknown evidence to handle these materials safely. Evidence from these cases may contain unknown materials that may present flammable, contact, and/or inhalation hazards in addition to any toxic effects. Special attention to the possibility of biological, nerve, or other toxins should be considered. Acids and bases may be encountered as evidence. These are very corrosive and eye and skin protection must be used. In addition, acids may be very reactive with chlorates, acetone, flammable liquids and water. Extreme care must be taken when mixing these compounds.

Tear gas products are irritants, by definition, and will cause physical discomfort if inhaled. If working with spray products or clothing items containing high concentrations of tear gas products, perform analysis in a fume hood and avoid contact with skin and eyes.

Inadequately packaged samples need to be repackaged and defective or damaged containers repaired or replaced.

As in all cases, care must be taken to obtain as much advance information about the submitted item(s) as possible. The most useful information will often come from case investigators and their scene reports.

Medical information regarding victim symptoms may be valuable, but it might be difficult or impractical to obtain due to HIPAA and confidentiality issues.

Due to the hazardous nature of many samples, it is recommended that scientists decline if possible to open evidence containers in a court of law or other public environment without thoroughly explaining the possible risks involved and the protective measures required before proceeding.

33 GLASS

33.1 INTRODUCTION

Fragments of broken glass are frequently found as trace evidence in a variety of types of crimes. Possible sources of glass evidence include but are not limited to vehicles, containers, windows and doors. Investigators should be encouraged to preserve and collect potential glass evidence, and to also collect appropriate known glass samples for comparison purposes.

This method covers the procedures intended for use by the trained examiner for the collection, documentation, and examination of glass evidence. The procedures include techniques for the characterization of glass and comparison of those fragments of unknown origin to known glass. The information gained from characterization of glass samples can be compared to reference databases in an attempt to identify the glass type or, more commonly, for comparison of fragments from a known source to fragments recovered as evidence. Additionally, the direction of force, the nature of the breaking force, and/or the sequence of impacts can sometimes be determined in non-tempered windows and car windshields.

The particular methods employed by an examiner will depend upon considerations of sample size, information desired from the analysis, applicability of a given technique to the sample type, and available laboratory equipment. Methods should be chosen based on ability to provide information about the possible origin of the material in question or based on ability to discriminate known samples from questioned samples. Particular consideration should be given to preservation of evidence when employing techniques which are destructive in nature. If an analyst discriminates the questioned glass and the known glass at any point in the chosen analytical scheme, no further examination is required.

33.2 ADVANTAGES AND LIMITATIONS

Glass is characterized by a number of physical features and elemental composition. The physical characteristics may include color, thickness, surface features, fluorescence, and refractive index. These features can be determined and evaluated by a variety of macroscopic, microscopic, and instrumental methods. The information obtained from these determinations can be used to provide investigative information or to compare known and questioned samples.

The analysis of glass may be limited due to sample size, heterogeneity of glass products within a single source, sample collection at the scene, sample handling and transportation.

33.3 APPARATUS AND EQUIPMENT

Glass Refractive Index Measurement (GRIM) and a variety of standard laboratory instruments and supplies are needed in the analysis of glass.

33.4 PROCEDURE

33.4.1 <u>CASE APPROACH</u>

Glass fragments should be cleaned to remove dirt, grease, and other debris prior to performing analytical methods in which the outcome could be affected by the foreign materials on the glass surface.

If sample size is limited, nondestructive methods must be exhausted before subjecting the sample to any destructive testing. Whenever possible, a portion of the evidence sample will be preserved for possible re-analysis. If consumption of the sample is a concern, slides used for GRIM analysis may be repackaged and returned to the agency or the crushed glass may be retrieved from the silicone oil and repackaged for return. However, in most cases, GRIM slides will not be retained or preserved after analysis.

The following criteria should be considered in the decision regarding the number of particles to test at each stage of the examination:

• The quantity of evidence available and the destructive or non-destructive nature of the testing
• The degree of similarity exhibited among the questioned samples during analysis
• The number of questioned particles consistent with the known standard(s)
• The type of glass (if known)
• The degree of variation noted within the known standard samples submitted

In cases involving an association, visual, elemental, and refractive index tests are considered a minimum. If the minimum examinations are not performed, the analyst must document the reason in the case notes.

In cases where the possibility for a physical match exists, samples should be evaluated for the presence of a physical match before any sample manipulation that might cause damage to the edges is performed. Care should be taken during side-by-side examination to clearly designate the known from questioned samples. If there are no corresponding edges or the possibility of a physical match does not exist for a particular sample, the examiner should proceed with compositional comparison.

33.4.2 ANALYSIS

Initial examinations

General trace recovery methods may be used to collect glass particles. These methods are outlined in the Trace Evidence Recovery chapter of this manual.

Note the condition of the evidence. Evaluation of any surface contamination should be done prior to cleaning or other analyses. If analysis of the surface contamination is needed it should be removed prior to glass characterization and testing. Glass fragments may be cleaned to remove dirt, grease, and other debris prior to performing analytical procedures. The type of contamination will dictate the cleaning method (i.e., soap and water, alcohol rinse, sonication, etc.). When cleaning glass fragments, be aware that cleaning with nitric acid may remove a manufactured coating.

If both original surfaces of a flat glass sample are present, use a micrometer to measure the thickness of the glass. A range for the thickness of the known flat glass should be determined if possible. Specular reflection may be useful in identifying original surfaces. Because thickness measurements are used for comparative purposes, the same micrometer should be used for known and questioned fragments of glass.

Note the color of the glass. Large fragments of glass are easily compared side by side. Slight color variations can be difficult to visualize in small fragments. Fragments of similar size and shape should be utilized when comparing color.

Stereomicroscopy can be used to help locate suspected glass particles. It is also used to characterize glass particles once recovered. Polarized light may be used to distinguish between glass particles and other particles such as quartz and plastic.

Glass should be examined to determine (if possible) the type of glass. Tempered vs. non-tempered (fragment size, shape and edge characteristics), windshield (laminated), container (curved surface), and windowpane (flat or float) all have shapes or features that might aid in identification/comparison.

Alternate Light Sources (ALS)

Glass fragments can sometimes be characterized by means of alternate light sources. The original surface should be examined for surface fluorescence with a shortwave UV light (254 nm). If the glass fragment was manufactured by the float method, the surface that was in contact with the molten tin bath will fluoresce a pale yellow with this UV wavelength. On small glass fragments this observation may need to be made using a stereomicroscope. Unless otherwise indicated, documentation of this test will mean that shortwave UV light of approximately 254 nm was used.

Other wavelengths of light may be used to observe and compare known and questioned glass fragments. If this examination is performed, the wavelength(s) used and the observations made must be documented in the case notes.

X-Ray Fluorescence Spectrometry

Refer to the XRF chapter of this manual for general procedures related to the use of XRF. Additionally, any XRF instrument used for semi-quantitative glass measurements will comply with additional Quality Assurance measures as outlined in ASTM E2926-13 Section 8.2.

The excitation voltage, tube current, process time, spot size, and collection time will be sample-dependent and left to the discretion of the examiner. Unless otherwise noted all samples will be run under vacuum. Fragments of known glass will be similar in size, shape, surface and thickness to the questioned glass fragments analyzed via this method, unless the samples are insufficient to do so

The preferred sample prep for glass fragments is to mount samples using a sample cup with x-ray-transparent film and adhesive. A removable craft adhesive such as TOMBOW® removable monoadhesive (or equivalent adhesive free of elemental interferences) is suitable. Samples should be mounted to present as flat a surface as possible to the x-ray beam. It may be necessary to reorient small irregularly-shaped glass particles to minimize any potential scattering effects, or to collect several spectra at different orientations.

For each known glass, 9 replicate spectra will be collected unless an insufficient number of fragments are submitted. For each questioned particle, 3 spectra will be collected if the fragment size allows.

GRIM (Glass Refractive Index Measurement)

The immersion oil selected should correspond to the anticipated refractive index of the glass. Locke silicone oil B is usually used since most glass will measure in this range. In order to prevent the possibility of contamination of the stock bottles of the silicone oils, it is recommended that an aliquot of oil be taken from the stock bottle of oil for use in a given case. The aliquot should be disposed of when all analyses for the case are completed, or used for non-casework GRIM analysis.

Following the instrument manufacturer’s instructions, appropriate measurements will be made of known and unknown glasses, and the refractive index values compared.

It is unusual for well-selected glass edges to produce aberrant data; however, surface debris, extreme internal stress, coatings or original surfaces may generate a refractive index value that is unrepresentative of the main body of the glass. Care should be taken to not be overzealous in the rejection of data; glass will always show a range of refractive index values. Efforts should be made to measure the entire RI range presented by the glass.

When possible with the evidence submitted, a minimum of ten slides representing ten separate pieces of a single object of known glass will be prepared and the refractive index will be measured for at least three edges per slide. If the known glass provided is inadequate for this number of slides, do the maximum number practical keeping in mind that statistical analysis is less dependable for small sample sets. The average RI and standard deviation for these known glass fragments will be calculated.

One slide should be prepared for each questioned fragment to be analyzed and a minimum of three data points collected (from three discrete edges).

Annealing

Annealing glass in casework analysis involves measuring the refractive index of a glass both before and after a controlled heating/cooling process. Annealing may add discriminating power with samples that are indistinguishable through other means. It may also be done to “reset” glass that has been exposed to extreme heat conditions.

Annealing can also be of help in determining glass type, where no known standard is available. The confidence with which these evaluations may be made is dependent on the experience of the examiner and the available reference collection data.

To perform annealing, each fragment will be broken in two. One piece will be analyzed by GRIM while the other half is first annealed and then analyzed by GRIM. The change in refractive index will be calculated and these values compared for known and questioned samples.

A sample annealing procedure is: Place small fragments of each glass on a ceramic spot plate or separate crucibles and position in the center of a muffle furnace. Increase the temperature to 600° C and hold at that temperature for 30 minutes. After 30 minutes, unplug the furnace and leave the chamber door closed to allow cooling to room temperature to occur slowly (approximately 10 hours).

Direction Of Force Analysis And Impact Sequencing

If directionality or impact sequencing analysis is requested, the overall pattern of fracture or fracture features on the edges of specific broken pieces of glass are evaluated and documented.

Based upon the type of glass that is broken, it may be possible to make some determinations as to type of force that produced the break, where the breaking force was applied, direction of force that broke the glass, and the sequence in which multiple forces were applied. Impact fractures in tempered glass are often not possible to evaluate due to the numerous small glass cubic fragments produced.

33.4.3 NOTE TAKING

Notes should include the source (as best known) of each glass sample, any sample treatment or preparation steps taken, results of visual and microscopic observations, results and data from any instrumental analyses. Refer also to the technical procedures for Trace Evidence Recovery and the QOM.

Measurements used only for comparative purposes are not considered critical measurements; tools such as micrometers do not need to be calibrated or verified by an external provider. However, the measurements for known and questioned samples should be made using the same measuring device.

Glass examinations utilize measurements for comparison and classification of glass fragments. These measurements are of two types - thickness and refractive index. The uncertainty of these measurements does not have a significant effect on the outcome of the analysis as the results are used primarily for comparison purposes. Classification range values of glass types far exceed the uncertainty of the measurements generated during thickness and refractive index analysis.

33.4.4 PACKAGING

Precautions should be taken to prevent cross-contamination between known and questioned samples. Known and questioned samples should never be packaged together and all packaging should be sealed to prevent leakage or contamination.

33.5 INTERPRETATION AND REPORTING

33.5.1 X-RAY FLUORESCENCE SPECTROMETRY

In cases where only questioned samples are submitted for glass type determination, the elemental composition may be used to assist the examiner in this determination. Levels of magnesium and iron may be especially helpful in distinguishing flat glass from container glass.

When comparing samples, the relative intensities of the elements in the known and questioned samples should be consistent if they could have come from a common source. Intensities of certain elements may vary slightly within a given glass object, or the size and/or orientation may affect the intensities detected. For glass comparisons, a statistical comparison using elemental ratios of relative peak intensity is used to minimize this sample- and measurement-related variation.

Peak intensity ratios are appropriate when spectral comparisons do not discriminate two samples and when there is a sufficient known glass sample. Calculating peak intensity ratios when visual spectral comparisons *do* discriminate is at the discretion of the examiner. Use of ratios for evaluation will follow the guidelines set forth in ASTM E2926-13.

Interpretation of Ratio Comparisons

Comparison of peak intensity ratios can occur in two ways: range overlap and mean ± 3 standard deviations. The choice of comparison method is case dependent and is at the discretion of the analyst.

Range Overlap

For each elemental ratio, compare the range of the questioned sample replicates to the range from the known sample replicates. If the ranges of one or more elemental ratios in the questioned and known sample do not overlap, it may be concluded that the samples are not from the same source.

± 3 Standard Deviations

For each elemental ratio, compare the average ratio for each questioned fragment to the average ratio for the known sample ± 3 standard deviations. To use an element ratio for comparison, the RSD (relative standard deviation) of that element ratio calculated for the known sample must be less than 15% unless there are extenuating circumstances documented in the case notes. If one or more elemental ratios of a questioned fragment do not fall within the average ratio for the known sample ± 3 standard deviations, it may be concluded that those samples are not from the same source.

33.5.2 GRIM DATA

Care should be taken to evaluate not just the mean of the data but the overall distribution of values and any potential outliers that may unduly affect the mean and standard deviation.

An analyst may choose to conduct statistical evaluation for potential outliers and reject data that is deemed statistically aberrant. One such test for this determination is the Grubbs test. The analyst’s notes must include data both before and after rejection of the data point(s), as well as documentation of the method of statistical evaluation

The refractive index for each questioned fragment will be compared separately to the refractive index range for each known glass. The range for a known glass is calculated using ± 2 standard deviations from the mean of the results. If the questioned fragment falls within two standard deviations of the average of the known glass, the known and questioned glass may share a common origin. If the refractive index of a questioned fragment falls outside of those boundaries (i.e. 2 SD of known), then the particle is reported as dissimilar to the submitted known glass.

If the known glass provided is not adequate (does not sufficiently represent the broken object), the range (average ± 2 SD) may not be useful for comparison. Assessment of the adequacy of the known glass will be at the analyst's discretion based on the type of glass, quantity and quality of the fragments provided, the amount of variation observed, scene details, etc. If the known glass standard is deemed inadequate or in some way requires modification of the 2 SD discrimination criteria, decisions will be documented in the case notes. Other methods of comparison may include range overlap or a defined range such as average ± 0.0002 RI units.

Direct refractive index comparison may not be appropriate for glasses with a different thermal history, as exposure to heat can alter refractive indices. For example, comparison of glass collected from a fire scene to broken shards found on a suspect's shoes may be comparing glass before extreme heat exposure to glass after extreme heat exposure. In these cases, the thermal history of the glass may be "reset" by annealing, allowing for better comparison. If post-annealing refractive index values are significantly different for known and questioned fragments of glass, they are discriminated and are reported as dissimilar.

33.5.3 DIRECTION OF FORCE ANALYSIS AND IMPACT SEQUENCING

Interpretation of the direction of glass fracture and fracture sequencing is based on physical characteristics observed visually or with the aid of stereo microscopy. These features may include fracture characteristics of radial cracks, cratering, and defects to lamination layers in vehicle windshields.

33.5.4 REPORTING

When only questioned samples are provided and the aim is to assist with investigative information, the examiner's report should include, if possible, the type and/or possible source of the glass and any other significant features of the glass (freshly broken, color, etc.)

When the examiner has concluded that the questioned samples are not consistent with the known samples, the report will state in effect that the questioned samples could not have originated from the item/object as represented by the known sample. When an exclusion has been made, the examiner should provide any information gained during the analysis of the samples which would be useful for investigative purposes.

When all analyses performed indicate that the questioned samples are consistent with the known samples provided, the report will state in effect that the questioned samples could have originated from the item/object represented by the known sample. The examiner may also add information regarding alternative sources for material of the type identified.

The examiner may choose to report that a meaningful comparison could not be made in some situations, including but not limited to the following:

- A high level of variability in the questioned samples and/or controls prevents comparison
- The manner in which samples were deposited, collected, or packaged prevents the examiner from acquiring a sample that is uncontaminated
- The quantity of sample provided is insufficient for analysis.

33.6 QUALITY ASSURANCE

Instrument calibration and maintenance will be performed on a regular basis, as outlined in each instruments' technical procedure. Calibration reports and maintenance are documented and can be found with the respective instruments.

Quality check results will be evaluated. If the results are not adequate for the instrument, equipment, or technique, then the instrument, equipment, or technique is not to be used until appropriate action steps are taken to correct the problem. A repeated quality check that yields satisfactory results deems the instrument, equipment, or technique appropriate for use.

It is essential to ensure that data collected by any analytical method is not subject to confusion or misinterpretation resulting from contamination of the case sample.

The scientist should take care to employ proper methods of cleaning tools, laboratory equipment, and work areas between samples in order to prevent contamination.

33.7 SAFETY

Care should be used when handling glass. Eye and body protection should be worn whenever glass is being broken.

Safety precautions for each analytical technique are outlined in the appropriate technical procedure manuals.

34 HAIR

34.1 INTRODUCTION

Hairs are frequently found as trace evidence in a variety of types of crimes. Hairs are examined microscopically for many characteristics, including growth stage, root and tip condition, general form, length, color, pigmentation pattern, medulla appearance, etc. These characteristics may be used to reach conclusions on whether or not a hair is suitable for DNA analysis and other investigative information.

Examinations for the suitability of a hair for DNA analysis include if a material is a hair, if a hair is of human or animal origin, the growth phase of a human hair, and the presence or absence of adherent follicular tissue. Hairs examined for suitability for DNA analysis are placed into one of four general categories: a hair fragment; a hair with an inactive growth stage root with no adhering cellular material; a hair with an active growth stage root with no adhering cellular material; or a hair with root that has some form of adhering cellular material.

Examinations for other investigative information include whether a human hair originated from certain body areas, presence of damage (burned, crushed, glass cut, insect, etc.), the presence of a hair disease, natural color alterations (dyed or bleached), indications of forcible removal, putrid roots, and the presence of additional trace evidence or biological materials (blood, semen).

34.2 ADVANTAGES AND LIMITATIONS

Hair examinations consist of microscopic analyses of the morphological characteristics. Such characteristics can be used to determine the suitability of a hair for DNA analysis as well as other investigative information. Due to the nature of hairs, examinations may sometimes be inconclusive about a particular characteristic.

Technology for both nuclear and mitochondrial DNA analysis continues to advance in its sensitivity in obtaining an interpretable genetic profile. Determination of whether a hair will or will not be analyzed by nuclear or mitochondrial DNA analysis is left to the discretion of the DNA section.

This laboratory system does not conduct microscopic hair comparisons for the purpose of identification of a contributor of a hair sample. This laboratory does not classify hairs based on anthropological categories for the purpose of racial identification.

34.3 APPARATUS AND EQUIPMENT

Basic laboratory supplies and microscopes are required for the examination of hairs.

34.4 PROCEDURE

34.4.1 <u>CASE APPROACH</u>

When receiving hair evidence, check the evidence packaging for the presence of damage and/or inadequate seals, making a notation of any discrepancies or other issues.

Loose hairs should be collected from an object by picking them off individually. Hairs that are embedded in or adhering to a person or object must be carefully inspected before removal. If appropriate, the location of these hairs should be carefully documented. Care must be taken not to contaminate, crush, or break the hairs.

The remaining hairs can be collected from clothing, bedding, or other large surfaces by scraping or lifting. Be aware that the adhesive from the lifting material could interfere with the analysis of surface treatments that might be present on the hairs.

Case scenario information may be used to search for or target specific types of hairs (e.g. searching only for pubic hairs, searching only for hairs with roots with tissue or an active growth stage, searching for forcibly removed hairs, etc.).

In cases where there is a large number of questioned hairs that either have tissue or an active growth stage, the hair examiner may use a comparison microscope to screen the questioned hairs against the range of characteristics observed in a control hair sample. Control hair samples must be plucked. Whole length plucked hair controls should be sufficient in numbers and quality and should represent the entire range of characteristics of the source.

Materials adherent on hairs (e.g. blood or debris) should be preserved.

34.4.2 ANALYSIS

Macroscopical and stereomicroscopical examinations may be used to determine color, length, shape, and texture. Hairs mounted in an appropriate medium may be observed with transmitted light microscopy to determine the internal microscopic characteristics of hair.

Depending on the case circumstances, hairs may be mounted in temporary mounting media (e.g., xylene or xylene substitutes) or semi-permanent mounting media (e.g., Cytoseal™, Entellan ®, Permount®, and/or similar products). A small about of xylene or xylene substitute should be used when removing hairs from an adhesive (e.g. from a lift or from a sticky note) to prevent loss of adherent trace materials and/or tissue.

If hairs are requested to be removed for DNA analysis, review the "Recovering Slide-Mounted Hairs or Semen Smears" chapter of the "Casework STR Analysis Procedures" manual from the DNA Section. Coordination between the hair examiner and the DNA analyst to decide an appropriate analytical strategy is recommended. If the hairs are to be transferred to a non-WSP laboratory for DNA analysis, the laboratory should be contacted for information as to their preferred method for packaging and shipment.

If the microscopic examination indicates that certain hair roots are suitable for nuclear DNA analysis then a photograph of each root is recommended to be taken and included with the casework documentation.

Human hair can be distinguished from other animal hair by examining features such as scale pattern, medulla, root, color, hair length, and/or shaft configurations.

The somatic origin of a human hair can usually be established by considering features such as length, cross-sectional shape, shaft configuration, medullary configuration, texture, taper, and appearance of the root. The types may include head, pubic, facial (beard or mustache), torso (chest, axillary, back, abdominal), limb/body, and eyebrow/eyelash hairs. Some hairs may have a range of features that cannot be categorized into one of these groups. Such hairs may be "transitional hairs" (those growing between two body regions). The somatic origin of such hairs may be noted as inconclusive, transitional hairs, list multiple categories, or a user defined catch all phrase. An example of a catch all phrase is "coarse body hair", defined as having some pubic and/or torso features.

By their nature, human hair fragments may be missing some of the features used to determine somatic origin. Therefore, somatic origin should not be determined for hair fragments, especially shorter hairs (those hairs roughly less than one inch) except when there is an investigative need and sufficient characteristics are present to make that determination.

34.4.3 NOTE TAKING

For hair examinations one should provide appropriate documentation in the notes for determination of: whether the hair is animal or human; fragment or non-fragment; length, color; general hair form; and root condition. One should also note other variable characteristics observed such as: color treatment, somatic origin, and damage.

34.4.4 PACKAGING

All items should be securely packaged when the examination is complete. Loose hairs should be stored in anti-static plastic zip-lock bags, paper folds, or glassine envelopes. The use of Post-it notes or adhesive tapes for the collection or storage can be used where it is deemed appropriate or for the best preservation of the evidence.

Hairs mounted in ~~a~~ semi-permanent mounting media on glass slides should be placed in cardboard slide mailers or other packaging that will adequately protect the slides from breakage. Hairs mounted in a temporary mounting medium may be recovered from the slide and returned to the original packaging. The slide may be discarded unless adhering material from the hair remains in the mounting media. In that case, the temporary mounting medium will be allowed to air dry, and the slide will be packaged in a cardboard slide mailer to preserve any possible evidence. Such preservation may include using clear tape or clear nail polish to keep the coverslip from moving on the slide.

34.5 **INTERPRETATION AND REPORTING**

A hair report should communicate if the hair is animal or human, root present or fragment, approximate length, color, and root condition. One should also report if there were any uncommon characteristics observed. For all hair examinations, any additional investigative information such as the type of damage or the type of trace materials adhering to hairs should be reported.

If the evidence is determined not be a hair, then the report should include if it’s a fiber, possible tissue that may be of interest for DNA analysis, or give a description of the material. The description of the material may be based on appearance (e.g. white strand with some surface texture) or a classification used in general criminalistics analyses (e.g. botanical debris).

If there are multiple hairs in an item of evidence, one may need to consider the case circumstances and what information needs to be communicated to the agency and to the DNA unit. Hairs can be separated as to their general observable characteristics. Any hairs that share similar observable characteristics can be “grouped” together in the report. By doing so, this does not mean that these hairs came from a single source, but the hairs are only being sorted according to the hair characteristics.

Microscopic hair comparisons are not performed in the laboratory. For cases in which hair controls are submitted and examined, no absolute inclusion statements will be made attributing questioned hairs to an individual(s) as a source based on microscopic examination. An individual may at times be excluded as a possible source of a questioned hair due to gross differences in color, morphology and internal structures.

The following statements will be generally used in laboratory reports when describing the suitability for DNA analysis:

34.5.1 FOR A HAIR FRAGMENT:
Mitochondrial DNA analysis may be a suitable approach for further testing of human hair fragments. Please consult with the DNA section to determine the best approach for this (these) hair(s).

34.5.2 FOR A HAIR WITH AN INACTIVE GROWTH STAGE ROOT WITH NO ADHERING CELLULAR MATERIAL:

Mitochondrial DNA analysis may be a suitable approach for further testing of the human hair with an inactive growth stage root with no adhering cellular material. While nuclear DNA analysis may be attempted, these types of roots often do not result in the generation of an interpretable DNA profile. Please consult with the DNA section to determine the best approach for this (these) hair(s).

34.5.3 FOR A HAIR WITH AN ACTIVE GROWTH STAGE ROOT WITH NO ADHERING CELLULAR MATERIAL:

Nuclear DNA and mitochondrial DNA analysis may both be suitable approaches for further testing of a human hair with an active growth stage root and no adhering cellular material. Please consult with the DNA section to determine the best approach for this (these) hair(s).

34.5.4 FOR A HAIR WITH ROOT THAT HAS SOME FORM OF CELLULAR MATERIAL:

Nuclear DNA and mitochondrial DNA analysis may both be suitable approaches for further testing of a human hair with a root and adhering cellular material. Please consult with the DNA section to determine the best approach for this (these) hair(s).

34.6 QUALITY ASSURANCE

Adequate literature references and reference material collections should be available.

34.7 SAFETY

Hair samples may constitute biohazards and appropriate precautions should be taken.

35 PAINT AND POLYMERS

35.1 INTRODUCTION

Searching for differences between questioned and known samples is the basic thrust of forensic paint analysis and comparison. However, differences in appearance, layer sequence, size, shape, thickness, or some other physical or chemical feature can exist even in samples that are known to be from the same source. A forensic paint examiner's task is to assess the significance of any observed differences. The absence of significant differences at the conclusion of an analysis suggests that the paint samples could have a common origin. The strength of such an interpretation is a function of the type and/or number of corresponding features.

In cases involving only questioned samples an attempt should be made to determine the composition and possible origin(s) of the material.

If the purpose of the examination is to determine a possible suspect vehicle year/make/model from paint found at a scene or from a victim, then the observed characteristics should be entered as a query in the Paint Data Query (PDQ) database from the Canadian RCMP.

This section describes techniques intended for use by the trained examiner for the collection, documentation, and examination of paint/coatings evidence. It also describes methods to develop discriminatory information using an efficient and reasonable order of testing. Possible sources of paint evidence include but are not limited to vehicles, buildings (interior and exterior painted surfaces), tools, appliances, cosmetics, and roadways. The particular methods employed by an examiner will depend upon considerations of sample size, information desired from the analysis, applicability of a given technique to the sample type, and available laboratory equipment. Methods should be chosen based on ability to provide information about the possible origin of the material in question or based on ability to discriminate known samples from questioned samples. Particular consideration should be given to preservation of evidence when employing techniques which are destructive in nature.

Analysis and comparison of polymeric materials other than paint, such as plastics and rubber, may be performed using the same techniques described herein. Identification of a polymer may be achieved by comparing instrumental data from FTIR or pyrolysis GC/MS analysis, to known polymer reference materials.

35.2 ADVANTAGES AND LIMITATIONS

Paint films are characterized by a number of physical and chemical features. The physical characteristics may include color, layer sequence and thickness, surface and layer features, contaminants and weathering. Chemical components may include pigments, polymers, additives and solvents. These features can be determined and evaluated by a variety of macroscopic, microscopic, chemical, and instrumental methods. The information obtained from these determinations can be used to provide investigative information or to compare known and questioned samples.

35.3 APPARATUS AND EQUIPMENT

A variety of standard laboratory instruments and supplies are needed in the analysis of paint and polymers. Unique to this type of analysis are the following:

- Modeling clay
- Carnauba wax
- Epoxy-type embedding medium
- Microtome
- Grinding and polishing apparatus
- Munsell Book of Color

35.4 PROCEDURE

35.4.1 CASE APPROACH

Paint is preliminarily identified as a thin film of usually pigmented material. It may be received as a smear on an item; scraped material; a layered chip or partially layered chip; or adhering to metal, wood, plaster, or other material as an intact coating. Visual and stereomicroscopic observation of the sample can often distinguish paint from other colored substances such as plastic or glass. The identification and collection of suitable samples is a critical step in paint analysis.

The potential for physical matches between known and questioned samples must be considered before selecting the method of paint sample collection. Care should be taken to preserve the potential for a physical match.

Questioned samples include all loose or transferred paint materials. Sources of questioned samples can include tools, floors, walls, glass fragments, hair, fingernails, roadways and adjacent structures, transfers or smears on vehicles, or transfers to or from individuals or clothing. Whenever possible, items with paint transfers should be appropriately packaged and submitted in their entirety for examination. When paint evidence is recognized, every effort should be made to manually remove it before using tape lifts to collect other types of evidence. If paint is collected with tape lifts, one should be aware of the possible difficulty encountered when attempting to manipulate paint samples bearing adhesive residues. In addition, components of the adhesive could contaminate the paint sample and change its apparent chemistry.

Smeared transfers can exhibit mingling of components from several layers or films that could preclude application of some of the analytical methods discussed in this section. Due to the difficulties associated with collecting smeared or abraded samples, the entire object bearing the questioned paint should be submitted to the laboratory whenever possible. When contact between two coated surfaces is indicated, the possibility of cross-transfers must be considered. Consequently, samples from both surfaces should be collected if available.

When feasible, known paint samples must be collected from areas as close to, but not within, the point(s) of damage or transfer as possible. These damaged areas are usually not suitable sources of known samples unless a fracture match is likely. The collected known samples should contain all layers of the undamaged paint film down to, and including, the substrate. Substantial variations in thickness and layer sequences can exist over short distances on painted surface. This is particularly true in architectural paint and automotive films where the curves, corners, and edges are often impact points and may have been subjected to previous damage, sanding or over-painting. If necessary, several known paint samples should be taken to properly represent all damaged areas. Known paint samples collected from different areas should be packaged separately and labeled appropriately.

Paint flakes can be removed from the parent surface by a number of methods. These include, but are not limited to, the following: lifting or prying loosely attached flakes, cutting samples of the entire paint layer structure using a clean knife or blade, or dislodging by gently impacting the opposite side of the painted surface. When cutting, it is important that the blade be inserted down to the parent surface. It should be noted that no one method of sampling should be relied upon exclusively.

Items submitted for analysis should be examined appropriately for trace evidence (including paint), as the first step in the analytical procedure. Any paint recovered should, at minimum, be examined visually and stereomicroscopically for color and layer structure.

Paint analysis generally begins with appropriate nondestructive tests. If the initial tests are inconclusive or not exclusionary, the examination may proceed with additional tests that are selected on the basis of their potential for use in evaluating or discriminating the samples of interest. For any given comparison, not all the techniques listed are necessarily required. Sample size, condition, and layer structure complexity should be considered when determining which techniques to use. The forensic paint examiner should always use the more specific and least destructive tests prior to those that require more sample preparation or consumption.

35.4.2 ANALYSIS

A reasonable scheme for forensic paint examinations is outlined below, with a more detailed description of the methods and instrumentation following. Samples that are neither constrained by amount nor condition should be subjected to analyses that will determine the color and texture of the paint as well as the number, order, colors and textures of the layers in a multi-layered sample. In most cases, instrumental techniques should be employed to analyze and compare both the pigment and binder portions of the sample. A combination of techniques, which provide discrimination between as many types of paints and coatings as possible, should be used. These techniques should also be selected to provide classification and/or component identification information to be used in significance assessments. The choice of techniques may change depending upon sample characteristics, such as the relative concentration of binder versus pigment, or available sample size.

Initial Evaluation
The initial evaluation should begin with a critical review of the case background information to determine if the necessary samples have been submitted. Additional samples should be requested if needed. Further review should be made of the samples' chain of custody, package sealing, identification markings, and any potential cross-contamination between samples. The original condition of the sample(s) must be documented. If the items are found to be suitable for further evaluation, a detailed accounting and description of the paint fragments and any co-mingled material should be documented. This involves describing the general condition, weathering characteristics, size, shape, colors, and layers present in each sample. This description can be accomplished by examining each item using a stereomicroscope. Occasionally, this can be the final step in an analysis if exclusionary features or conditions in the sample(s) are identified during the initial evaluation.

Written descriptions, sketches, photography or other imaging methods must be used to document each sample's characteristics. The goal is to produce documentation that will be meaningful to a reviewer in the absence of the recording examiner. The resulting notes must be sufficient to document the conclusions reached in the examiner's report.

Physical Match
The most conclusive type of examination that can be performed on paint samples is the physical matching of samples. This may involve the comparison of edges and surface striae between samples, or the comparison of surface striae on the underside of a questioned paint fragment to those of a parent surface. The edges and/or striae in question must possess unique characteristics.

If there are no corresponding edges, or the possibility of a physical match does not exist for a particular sample, the examiner should proceed with compositional comparison of both the binder and pigment portions of the paint evidence.

Sample Preparation and Layer Analysis
The layers in a paint film are identified by viewing sample edges at magnifications ranging between 5X and 100X. The more obvious layers are generally visible without sample preparation. Definitive paint layer identification usually requires sample preparation techniques such as manual or microtome sectioning and/or edge mounting and polishing. Samples can be prepared for observations by embedment and thin sectioning with a microtome, hand-sectioning or scraping with a scalpel, mounting in carnauba wax and cutting with a scalpel, mounting edge-on in modeling clay, or smearing the paint to a thin layer on a microscope slide. A combination of techniques may be required to fully characterize the layer structure. The extent of sample manipulation and preparation will depend on the amount of paint available, its characteristics and the properties the analyst wishes to observe.

Paint layer structure can be observed by using a scalpel blade to expose a fresh edge on the paint chip where the layers can be viewed. One method is to make an oblique cut through a sample. The larger surface area created by this angled cut may enhance layer visualization and assist in the detection of layer inhomogeneities. Individual layers may also be sampled more easily using this type of preparation. An alternative technique is to lay the scalpel blade at a low angle perpendicular to the edge of the paint and slicing a very thin cross section. The layering on the chip edge will be more clearly exposed and the thin cross section that is produced is also useful for further microscopic examination, if necessary.

Preliminary solvent tests can be conducted on the manually prepared sections and layer fractions.

Subtle differences in color, pigment appearance, surface details, inclusions, metallic or pearlescent flake size and distribution, and layer defects, may require microscopic comparisons of the edge, oblique cut and surface views of known and questioned paint samples. These comparisons must be carried out with both samples positioned side by side and in the same field of view. Various light sources should also be employed when evaluating similarities in color. Color comparisons made with a comparison microscope should be checked by exchanging the slides to the opposite microscopes and comparing again.

Cross-sections (embedded or thin-section preparations) may provide additional information as to the layer sequence, layer thickness, color, pigment distribution, pigment size, and composition of the individual layers that may not be possible to obtain with gross examination. Embedded preparations can be prepared by polishing and/or microtomy. Thin sections can be prepared using a variety of microtomy techniques. Examination and analysis of the cross-sections can be conducted using a variety of analytical techniques that may include light microscopy, UV-visible microspectrophotometry (MSP), infrared microspectrophotometry (FTIR), and electron microscopy (SEM/EDX) or X-ray Fluorescence (XRF).

Smears should be removed from the substrate before examination since the underlying surface may affect the perceived color. This may be accomplished by using a razor blade or scalpel.

Visual and Microscopic Techniques
Paint chips should be examined in their original condition and also in cross section. Cross-sections can be prepared manually or by using a microtome. Operation of the microtome apparatus is specific to the style and manufacturer. Refer to the manufacturer’s directions for the operation of a particular microtome.

Cross sections of samples can be mounted on a slide using mounting medium and a cover slip.

The following are some physical characteristics that may be noted either in the initial examination or in the cross section of the paint sample:

- Color - often the most discriminating feature
- General condition - size, shape, smeared, scrapings, peels
- Appearance with UV or other alternate lighting
- Layers - number, sequence, thickness, texture
- Pigments and fillers
- Inclusions - metal flakes, pearlescent pigments
- Texture - glossy, flat, rough, smooth
- Surface features – striations, abrasions, dirt
- Weathering characteristics
- Miscellaneous features - substrate, bubbles, etc.

Polarized Light Microscopy (PLM) is appropriate for the examination of layer structure and the comparison and/or identification of particles present in a paint film. These particles may include pigments, extenders, additives, or contaminants. Extenders and other components of a paint film are generally of sufficient size to be identified by their morphology and optical properties using this technique. Although some pigment particles are too small for definitive identification by this method, exclusionary features may still be evident between samples. Selected refractive index liquids may enhance the visibility of certain paint components or pigment particles by altering the contrast observed in a microscopic examination of prepared slides of paint smears.

In some instances, PLM may allow similar layers to be more easily distinguished in a paint cross section versus other lighting techniques. Suitable samples for examination by PLM include, but are not limited to, thin peels, thin sections, pyrolysis and low temperature ashing residues, sublimation condensates and dispersed particles in a solvent, oil or other mounting medium.

Epi-illumination can be useful for examination of cross sections, where reflected light can provide better visibility of heavily pigmented and opaque layers.

Microscopic Examination of Pigments
In order to examine pigments microscopically, the examiner should isolate the material to be viewed from all other layers and contaminants in the sample. Samples of the individual layers can be prepared by dissecting the sample using a scalpel. The top of the first layer should be scraped away and discarded as this layer can be contaminated with substances such as cleaning materials or waxes and can also be partially degraded due to exposure to the elements. To obtain samples of the individual layers, lay the scalpel blade at a low angle to the paint chip and slice thin sections from the top layer only. Continue downward through the paint chip obtaining thin sections of each layer. (This same technique can be used to obtain samples for microchemical/solubility tests, FTIR analysis, and X-ray analysis on individual layers.)

Once a sample of each layer has been obtained, the material is then transferred to a clean microscope slide; mounting medium is added; and a glass cover slip placed on top. With a rubber cuticle pusher or a pencil eraser move the cover slip in a circular motion while the mounting medium is soft. Once the material is dispersed in the mounting medium and any large aggregates are broken, allow the medium to dry.

Slides prepared as above should then be viewed using a microscope with bright field and polarized light microscopy. Samples can be viewed for comparison of known samples to questioned samples using a comparison microscope with bright field and polarized light microscopy. Microscopic information in combination with instrumental data (FTIR and/or X-ray) may be used for pigment identification provided that the appropriate pigment standards are available for comparison.

Microchemical/Solvent Methods
The use of various solvents and chemical reagents is based not only on dissolution of paint binders but also on pigment and binder color reactions with oxidizing, dehydrating, or reducing agents. Several schemes have been published to systematically discriminate between paint films of differing pigment and binder composition that are otherwise similar in visual and macroscopical appearance.

Solvent/microchemical tests are destructive and should be applied first to known samples in order to evaluate their efficacy to a specific case, and they should be used only in situations in which an adequate questioned sample is available.

Solvent/microchemical examinations should be applied to both questioned and known materials concurrently. Known and questioned samples should be compared under approximately the same conditions and concentrations. The effects of various tests are recorded immediately and then at reasonable intervals for the duration of each test. It is desirable to apply such tests not only to intact paint films, but also to peels of each individual layer to avoid interaction with neighboring layers and to observe the dissolution process more critically.

Reactions such as softening, swelling, curling or wrinkling, layer dissolution, pigment filler effervescence, flocculation, and color changes are some of the features that may be noted. The results of these tests are inherently difficult to quantify. Therefore, they are primarily used for preliminary classification and comparison.

To perform microchemical/solubility tests, one or two drops of reagent should be added to the sample on a glass slide or in a spot plate while viewing the reaction under the stereomicroscope. Reagents should be prepared from chemicals that are Reagent Grade or better. Samples from individual layers or a cross section of a chip may be used.

Infrared Spectroscopy
Because the paint fragments to be analyzed are often quite small, a beam condensing or focusing device is normally required, and a Fourier transform infrared (FTIR) spectrometer is recommended. Both transmittance and reflectance techniques may be used for the analysis of coatings, but in most cases, transmittance methods are preferred because all the sampling wavelengths are subjected to the same path lengths and most of the reference data of coatings, binders, pigments and additives consist of transmittance spectra. Attenuated Total Reflectance (ATR) spectra can be corrected for comparison to transmittance reference materials if necessary.

Certain types of coatings, including automotive undercoats and many types of architectural coatings (especially those with low luster finishes), usually contain significant amounts of inorganic pigments. These pigments tend to have most of their significant infrared absorptions in the lower frequency spectral regions, and several have all of their absorptions in the region below 700 cm-1. An FTIR spectrometer equipped with cesium iodide (CsI) optics and a deuterated triglycine sulfate (DTGS) detector can collect spectral data to 220 cm-1, although it requires longer analysis times than the standard potassium bromide (KBr) optics and the mercury cadmium telluride (MCT) detector.

If a multiple layer coating system is to be examined, optimal results can be obtained if each layer is isolated and analyzed separately. Methods that use solvents to assist in the sample preparation should be used with caution because they might alter the sample or result in the production of residual solvent spectral absorptions.

An infrared microscope accessory permits the analysis of a small sample or a small area of a sample. The infrared microscope accessory may also be used to sequentially sample individual layers of a multi-layer coating system that has been cross-sectioned. Generally, it is desirable to press such a sample after sectioning to produce a wider width for each layer and to produce a more uniform thickness. All spectra of individual layers should be examined to determine if absorptions of adjacent layers are contributing to the spectrum.

Generally, the microscope will provide the most satisfactory results for paint analyses with the least amount of sample. Analyzing samples on the FTIR bench will generally require a greater amount of sample, but there may occasionally be samples that are better suited to analysis on the bench (i.e., if samples are particularly opaque, not homogenous, or extended range spectra are necessary, etc.). If samples are to be run on the bench, the paint can be ground with KBr and pressed into a pellet, pelletized with a compression cell, analyzed with an ATR attachment, a diamond anvil cell (DAC), or other suitable sampling accessory.

Transfers of coatings resulting in smears on various substrates may be sampled in situ using an appropriate attenuated total reflectance (ATR) accessory. The substrate itself (assuming it is not a metal) should also be analyzed and any contributions from the substrate should be considered. If the substrate is a metal, or highly reflecting, it may be possible to obtain a reflection-absorption spectrum of the smear using the reflectance mode of an infrared microscope accessory.

It may not be possible to separate the layers in some cases. In these instances, bulk analysis is acceptable. The examiner should be aware that variability in the relative thickness of layers or the manner in which they were deposited may cause variability in the results obtained from bulk analyses. In these instances, the examiner may wish to take multiple spectra from each sample and examine the variability. It may also be possible to obtain spectra specific to the exposed layers of paint using ATR.

Microspectrophotometry
Microspectrophotometry can be used to discriminate the color of visually similar or metameric paint samples. Due to the typically small size of forensic samples, microspectrophotometry is often the best objective means for obtaining color data for paint comparison.

Transmission microspectroscopy of thin cross sections offers a more definite color analysis versus reflectance techniques. However, transmission microspectroscopy demands the most care in preparation. Although thin cross sections can be manually prepared, improved reproducibility can be achieved using a microtome. Consistency in surface preparation and thickness between questioned and known samples will give more accurate comparisons.

Diffuse reflectance can be used to examine the outer surfaces of paint films. Diffuse reflectance measurements of paint surfaces are affected profoundly by surface conditions such as weathering, abrasion, contamination, and texture. This fact can provide useful discriminating information when an examiner is faced with distinguishing different surfaces that were originally painted with the same paint formulation. Careful reference sampling is essential to the success of color comparisons of such surfaces.

Diffuse reflectance can also be used on the edges of cross sectioned paint layers much as it is on outer paint surfaces. Before analysis, questioned and known samples should be mounted side-by-side on edge and polished to a smooth surface using a polish of 3 micron grit size or less. Microtomed samples without surface defects may be used without polishing. The requirement for consistent surface finish characteristics for all samples is achieved easily if the known and questioned samples are mounted and prepared in a single mount.

Pyrolysis Gas Chromatography Mass Spectrometry
Pyrolysis GC/MS provides useful structural information about the binders in paint samples. Organic pigments may also contribute peaks in a pyrogram. Due to the sensitivity of the technique, differences in layer thicknesses and variations within non-homogeneous samples can result in differences in relative peak ratios of pyrograms.

Paint samples can be run in bulk (all layers together) or by single layer. When running bulk samples, care must be taken to ensure that relative layer thickness is consistent between samples. Care should also be taken to ensure that the top surface is free from contaminants, and that the bottom layer is free from substrate contamination. Multiple runs of the sample may help demonstrate minor variations in the pyrograms due to non-homogeneity and variations in layer thicknesses. Sample sizes should also be consistent from one run to the next.

Comparison of peak patterns of pyrograms between questioned and known samples is a powerful qualitative comparison tool for discriminating between samples; however it is not generally used in the identification of chemical components. Analysis of mass spectra of major peaks may be performed to gain some structural information about the polymer. Additionally, comparing mass spectra averaged over the entire TIC may be done to complement the comparison of pyrogram peak patterns.

Many automotive undercoats and architectural paints have high concentrations of inorganic pigments and extenders. Inorganic residue remaining after pyrolysis may be analyzed using PLM, XRF, and/or SEM/EDS to determine inorganic content.

Scientists must ensure that there is sufficient sample to perform destructive testing prior to conducting pyrolysis on evidence samples.

Scanning Electron Microscopy-Energy Dispersive X-ray Analysis (SEM-EDX)

Because of the relative inhomogeneity of paint samples on a microscopic scale, comparison of the composition of layers is generally performed by a non-quantitative method, such as direct spectral comparison or peak ratioing. Quantitative methods are not generally used for paint analysis. Elements in low concentrations may require longer collection times to be detected by SEM/EDX.

When results are obtained from samples prepared in cross section, either by microtomy or polishing, care must be taken to ensure that the EDS data generated are representative only of the paint layer of interest, or that any adjacent layer contributions are considered.

Sample preparation methods such as thin peels are preferred over stair-stepping methods. Stair step sample preparation allows larger areas to be analyzed and possibly avoids inhomogeneity concerns, but faces the potential for penetration into the underlying layer. If samples are prepared as single layer peels, the concerns of penetrating and/or sampling adjacent layers are avoided.

Mapping of elements across the cross section of a multi-layer paint can be useful for explaining or demonstrating elemental distributions and elemental associations. However, elemental maps are generally not quantitative and may lack the sensitivity to demonstrate minor sample differences.

The elemental composition of paint smears that cannot be lifted from a substrate can often be estimated by subtraction of the substrate's X-ray spectrum from the combined smear-substrate spectrum. However, co-mingling of the smeared paint with substrate surface contaminants, the low mass of the smear, and typical inhomogeneity of paint can produce significant deviations of the smear spectrum from that of the original paint.

"Atomic number contrast" images are produced in the SEM by the collection of backscattered electrons. These images are used to characterize and compare the structure of paints, including layer number, layer thickness, distribution and size of pigment particles, and the presence of contaminants.

X-Ray Fluorescence (XRF)
For paint analysis, typically a micro-XRF (µ-XRF) is used. XRF analysis is less spatially discriminating than SEM-EDX because of its larger analytical beam size and the greater penetration depth of X-rays compared to electrons. However, XRF has better sensitivity for most elements than SEM/EDX and can detect higher energy X-ray lines than can SEM-EDX. Because of the significant penetration depth of the primary X-rays, XRF analysis will generally yield elemental data from several layers of a typical multilayer paint fragment simultaneously. Since variations in layer thickness may cause variations in the X-ray ratios of elements present, this technique can be used only comparatively or qualitatively.

If a multiple layer coating system is to be examined, optimal results can be obtained if each layer is isolated and analyzed separately. Individual layers of a multi-layer coating system may be examined sequentially in a cross sectioned sample. Generally, it is desirable to press such a sample after sectioning to produce a wider width for each layer and to produce a more uniform thickness. All spectra of individual layers should be examined to determine if absorptions of adjacent layers are contributing to the spectrum.

It may not be possible to separate the layers in some cases. In these instances, bulk analysis is acceptable. The examiner should be aware that variability in the relative thickness of layers or the manner in which they were deposited may cause variability in the results obtained from bulk analyses. In these instances, the examiner may wish to take multiple spectra from each sample and examine the variability.

Make/Model Searches
In cases where only questioned samples are submitted and the sample is determined to contain the original equipment of manufacture (OEM) paint, the examiner may be able to provide information regarding the possible make, model and year of a vehicle. This may also be useful in situations where

the questioned samples are OEM finish and are not consistent with the known samples provided since the examiner may be able to provide helpful investigative information.

Using analytical data about the chemical composition of a sample, a make/model database search using the Paint Data Query (PDQ) software may be performed. Instructions for performing a search are outlined in the PDQ User Manual. This may require use of a Munsell Color Book to determine the color designations for the undercoat/primer color of the sample.

Once preliminary search results are obtained, the FTIR spectra of the questioned sample can be compared to the spectra of the PDQ spectral database "hits". At this point, it is also possible to make a visual comparison of color with the manufacturer color books for the appropriate make, model, and year.

If the examiner feels it is necessary to make a direct comparison between the questioned sample and the PDQ match, a sample of the PDQ paint match(es) can be requested from the FBI Trace Evidence Unit or the RCMP PDQ team who maintain and archive the PDQ paints.

35.4.3 NOTE TAKING

Notes should include the source (as best known) of each paint sample, any sample treatment or preparation steps taken, results of visual and microscopic observations, results of chemical tests and data from any instrumental analyses. Spectra should be evaluated to identify as many components in the sample as possible. A library spectrum or known reference data should be included to document the interpretation of the sample spectrum. Refer also to the technical procedures for Trace Evidence Recovery and the QOM.

Paint examinations and comparisons are qualitative techniques and as such do not utilize measurements that will have a significant effect on the outcome of the analysis. Measurements are occasionally taken to document the size of paint fragments but are not used to establish a critical value.

35.4.4 PACKAGING

Precautions should be taken to prevent cross-contamination between known and questioned samples. Known and questioned samples should never be packaged together and all packaging should be sealed to prevent leakage or contamination.

If the original packaging is not secure (wadded napkin, etc.), place it in an appropriate sealable package. Seal and mark the package with initials, lab number, item number, and date.

Paint collected in the laboratory should be sealed in a secure package and labeled as to what item it was collected from, the laboratory number, initials and date. Plastic bags tend to have static charges that may make handling of small fragments difficult; paper or metal containers may be better for such items. Small portions of the overall sample which are used for various portions of analysis will necessarily be destroyed in the process, while some portions (such as embedded chips) could conceivably be recovered. If this material represents a significant portion of the evidence, it should be returned as part of the evidence. If the embedded material is only a small part of the total, it may be considered expendable.

35.5 **INTERPRETATION AND REPORTING**

Many of the microchemical/solubility tests produce empirical color changes. These analyses are employed for comparative purposes only, and no conclusion is drawn regarding the chemical composition of the sample.

In the cases of other microchemical/solubility tests, the results can provide information regarding the sample composition. Guidelines for interpretation of these analyses are listed below.

- Acetone reactivity:

    - Enamels are insoluble in acetone.
    - Lacquers are acetone soluble.
- Chloroform reactivity:
    - Acrylic lacquers are chloroform soluble.
    - Nitrocellulose lacquers are insoluble in chloroform.
- Diphenylamine reactivity:
    - Diphenylamine reagent is a test for nitrates and nitrites. Paint containing nitrocellulose either as a binder or an additive will give a deep blue color reaction within a few seconds after addition of the reagent.
    - Note that other strong oxidizers may give positive reactions as well.
- Glacial acetic acid (hot):
    - Acrylic melamine dispersion (NAD) enamels are soluble in hot glacial acetic acid.
    - Acrylic melamine solution enamels and water-borne enamels are insoluble in hot glacial acetic acid.
- Xylene reactivity:
    - Acrylic dispersion lacquers are slowly soluble in xylene.
    - Acrylic solution lacquers are insoluble in xylene.

In order for two spectra to be considered consistent with one another, all significant data in the known spectra must be present in the questioned spectra. Conversely, all significant data in the questioned must also be present in the known.

In general, the relative intensities of the peaks in the known and questioned samples should be the same. A sample that is not homogeneous, particularly in pigment dispersion, can sometimes cause a situation where there is variability in the intensities of certain peaks even within a given sample. In this situation, the examiner may wish to take multiple spectra from a given exhibit to demonstrate variability of these peaks. If documentation of the variability is provided, the significance of differences in relative intensities between the questioned and known samples may be discounted when appropriate.

When only questioned samples are provided and the aim is to assist with investigative information regarding a possible source, the examiner's report should include, if possible, whether the paint is refinish or OEM for automotive samples, or the general type of paint for other paint samples.

When the examiner has concluded that the questioned samples are not consistent with the known samples, the report will state in effect that the questioned samples could not have originated from the item/object as represented by the known sample. When an exclusion has been made, the examiner should provide any information gained during the analysis of the samples which would be useful for investigative purposes. This may include but is not limited to information regarding possible origins of the material and whether the material is refinish or OEM.

When all analyses performed indicate that the questioned samples are consistent with the known samples provided, the report will state in effect that the questioned samples could have originated from the item/object represented by the known sample. The examiner may also wish to add information regarding alternative sources for material of the type identified, however, this is optional.

Although it may be useful to attempt identification of binder chemistry, pigments, and additives present in a sample and document these findings in the case notes, the reporting of information regarding the composition of the standards and questioned samples is left to the discretion of the analyst. There may be instances where binder classification is impossible or impractical due to heavy pigment loading of the sample.

The examiner may choose to report that a meaningful comparison could not be made in some situations, including but not limited to the following:

- A high level of variability in the questioned samples and/or controls prevents comparison;
- The manner in which samples were deposited or collected prevents the examiner from acquiring a sample that is uncontaminated (i.e., layers smeared together, cannot separate paint from substrate, collected on tape, etc.)
- The quantity of sample provided is insufficient for analysis.

35.6 QUALITY ASSURANCE

When collecting samples from a single layered paint or from the top layer of a multi-layered paint for instrumental analysis, care should be taken to avoid any oxidized material which may be present at the very top of the layer, any waxes or soaps which may have been used on a vehicle, and any debris which may be present on the sample.

Updates to the PDQ software are received periodically from the RCMP and should be installed promptly.

35.7 SAFETY

Single edged razors, scalpel blades and the use of a microtome to cross-section paint chips could cause cutting damage to fingers.

36 PHYSICAL MATCH

36.1 INTRODUCTION

Materials which may be submitted for a physical match examination can include broken glass (from burglaries, vehicle accidents, shootings, etc.), automobile parts from accidents, broken wood or metal (bats, sticks, architectural structures, etc.), paint chips, tape, wires, plastic bags, household items, and any other type of material/object that may be physical evidence in a criminal investigation.

Physical match examinations may not be initially requested by the submitting agency; therefore, prior to performing any chemical and instrumental analysis on such submitted items, the examiner should consider attempting a physical match. That is, to determine if the known and questioned items were, at one time, a single item. Coordination with the DNA and Latent Prints sections may also need to be considered before any work on a physical match examination is conducted. If the examiner obtains adequate identifying characteristics, a physical match can be made, thus an absolute identification between two (or more) items to the exclusion of all others.

Physical match examinations are conducted to physically fit evidentiary materials back together along available physical edges of the two or more items. When a physical match is accomplished, this examination provides conclusive evidence that the materials were at one time a single unit.

36.2 ADVANTAGES AND LIMITATIONS

The examination of items for physical match possibilities can provide important investigative information to the case such as providing a definite connection between the suspect and victim and/or the scene.

Putting a “jigsaw puzzle” together may seem to be a fairly easy analysis. However, a good background understanding of the various sub-disciplines of the materials analysis section and other sections within the crime laboratory system is necessary to assist the scientist in evaluating the evidence and determining:

- The feasibility of attempting a physical match with the type of material submitted,
- Whether the attempts at obtaining a physical match will damage or change the evidence and/or if additional evidence needs to be requested,
- If there is a need to contact a supervisor/experienced scientist from another section for additional advice or guidance about additional testing and/or the sequence(s) of testing before handling the item.

36.3 APPARATUS AND EQUIPMENT

Sample handling tools, packaging materials and a variety of microscopes are needed to perform physical match examinations. Individual case circumstances will dictate additional requirements and modifications.

36.4 PROCEDURE

36.4.1 ANALYSIS

In the preliminary examination and initial “fitting” of pieces together, it is extremely important to remember that the pieces should only be juxtaposed to each other and not physically connected to or touching each other. Prematurely “fitting” pieces together can alter and/or destroy fine tips, shards, detail, etc. on the edges of the pieces. These features are an important part of the examination and can add significant weight to the conclusions when they are present and “match” into the other piece. Documentation of such features is needed before the final “fitting together” is conducted and documented. Also, there could be an unintentional transfer of trace evidence between pieces if the pieces do not actually belong together.

Examine each item of evidence visually to determine the correct orientation of the pieces. Begin with matching a surface (e.g., the outer color coat of paint chips, the fluorescing surface of float glass pieces, the smooth or finished surface of plastic fragments). After the pieces are marked for identification and "sided", the contours, edges, colors, surface markings, etc. are used to help align pieces correctly. It is often helpful to closely fit together pieces of a given item to itself ("knowns") and then closely fit in the additional items ("questioned") in the case.

Observe all orientations of the fit. Scratches, stains and/or defects that traverse the broken, cut or torn edge serve to reinforce the physical fit conclusion.

Observe the matching sides on end so that the cross-section view is observable. Matching hackle marks and defects may be seen and serve to reinforce the physical fit conclusion.

Matching fabric pieces involves examination of general size and shape, weave, fiber type, twist, colors, long versus short threads, and thread counting.

When matching flexible materials (e.g., fabric, tape, and some plastics), care must be taken to account for edge rolling, stretching, and twisting. Use of double stick tape on a rigid plate may be useful to stabilize the edge during comparison and to reduce the effects of distortion from stretching or twisting.

It may be beneficial to make tic marks and/or lines on the different pieces of the physical match to enable a re-assembly of the pieces at a later date. These marks are only to be placed on an area of the pieces where no identifying features exist that support the physical match.

Depending on the case scenario, it may be useful to obtain crime scene photos, sketches, or other pertinent documentation from the submitting agency to assist in understanding where the submitted items were found.

A stereomicroscope with transmitted and incident light and/or a rolling stereomicroscope, all preferably with magnifications up to 100X, most likely will be utilized during a physical match analysis. A compound and/or comparison microscope may also be necessary for certain portions of the analysis or types of evidence.

36.4.2 <u>NOTE TAKING</u>

Photographs are to be taken of any pertinent edges and/or unique features and the fit between the edges of the items/pieces showing the physical match. Photos should also relate the location of the physical match to the overall item.

Close-up photographs with scale are necessary of the ends/edges of the pieces and of any material adhering to these areas.

Detailed descriptions and supporting sketches and/or photographs, especially macro, of the physical match will be in the notes.

Each of the above is to be documented within the case notes and should have legible markings for identification which are also described in the notes if not obvious from the photograph. These notations may be made directly on photocopies, photographs or sketches in the notes.

36.4.3 <u>PACKAGING</u>

The nature and condition of the items submitted will dictate the packaging. A different type of packaging method may be necessary after examination in order to retain the integrity of the area with the physical match.

36.5 INTERPRETATION AND REPORTING

Identification of a physical match is a cumulative effect of numerous features and characteristics in all three dimensions of the pieces/items being compared. Information from all three dimensions is needed to make a definitive conclusion of a physical match. These features and characteristics will be dictated by the composition and function of the item(s) being examined.

If a physical match is obtained, it can then be concluded that the particular piece(s) to the exclusion of all others were at one time a single item. Based on case circumstances, a physical match may provide significant evidence about interaction(s) between the single item and a particular place and/or person. Examples of this include comparisons where a paint chip found at a scene is fitted to a particular tool found in the suspect's possession or a piece of plastic is fitted to a damaged area of a vehicle suspected in a hit and run.

36.6 QUALITY ASSURANCE

Physical matches will be verified by a qualified examiner and documented in the case notes in accordance with the QOM.

Permanently mark and identify areas of the physical match on the submitted evidence items.

36.7 SAFETY

Take appropriate safety precautions when working with the ends/edges of items submitted for a physical match examination.

37 TAPE

37.1 INTRODUCTION

Tape evidence is found in a variety of criminal acts. Tape can be found in gags, bindings and ligatures, in explosive devices, on weapons, and on threatening packages or letters. The purpose of tape analysis is to determine whether or not a questioned sample can be associated with a known sample source. Tape analysis may also provide investigators information regarding the manufacturer when a source has not been identified.

Duct tape, packaging tape, electrical tape, and masking tape are the types most commonly found as forensic evidence. These tapes may be constructed of several layers of backing, adhesives, reinforcements, and release coatings. Each of these layers may be composed of a variety of materials such as rubber, polymers, paper, woven materials, films, pigments, organic and inorganic fillers, and metals.

Tape examinations include visual and microscopic observations of the layer structure, physical measurements of the tape, and details of fabric reinforcement construction. Chemical and instrumental methods are used to describe the chemical and elemental composition of the materials in each layer.

The technical procedures for the analysis of tape and synthetic polymers are adapted from the Scientific Working Group for Materials Analysis (SWGMAT) Guidelines for Forensic Examination of Pressure Sensitive Tape. Terminology associated with pressure sensitive tapes can be found in Appendix D: Pressure Sensitive Tape Terminology

37.2 ADVANTAGES AND LIMITATIONS

A physical match is the strongest association achievable with tape evidence. The demonstration of a physical match between questioned and known tape ends provides a significant link between these samples. In addition, physical matches between the ends of several pieces of questioned tape will define the relative position of the pieces on the tape roll. This description may provide reconstructive information for the investigator.

The techniques used for the examination of tape evidence can be applied to the analysis of other synthetic polymers, such as hard plastics, rubbers, elastic foams, rigid forms, and films. Automotive parts, reinforced hose, wire insulation, tubing, plastic bags, plastic wrap films, clothing, and plastic glitter particles are some examples of sources of synthetic polymer evidence.

The analysis and comparison of tape evidence can provide valuable information due to the variability of tape products. However, some classes of tape exhibit more variability than others. In general, the more complex the product (e.g., duct tape), the more variable it is. The common tape classes and their components can be found in Appendix E: Tape Construction and Classes. Studies have shown differences between randomly selected rolls of tape, but because of the ever-changing tape markets, suppliers, and economics, it is not feasible to establish the statistical probability that a given sample would have the same physical and chemical characteristics as a randomly selected tape.

While tapes within a specific class may appear similar on a macroscopic level, differences may be found on close analysis of the physical and chemical characteristics. Differences are readily observed in tapes manufactured in different plants.

Differences may also be found between batches of tape products within the same plant due to changes in raw materials and processing that occur over time. Also, the many components that comprise a given tape product are subject to supply-and-demand fluctuations in the market. For example, a lower bid for some minor component may lead to its substitution from one batch to the next, resulting in compositional changes that can be detected in the forensic laboratory. While it is less likely to find differences in tape rolls produced by the same production line, the probability of finding differences between batches increases with time between batches.

It may be feasible to detect physical differences between rolls of tape produced in the same batch. For example, one batch of duct tape produced in a large sheet may be slit into nominal two-inch wide (~50.8 mm) individual rolls. There are numerous cutters spaced along the width of the sheet that can result in slightly different roll widths within the same batch. Differences in the warp yarn offset from the machine edge may also be found in rolls from the same batch.

Within-roll variability has been assessed using different analytical instruments. No significant within-roll variations have been reported.

In the comparison of tape samples, much information can be obtained from macroscopic and stereomicroscopic examinations. Exclusions at this stage preclude additional analysis. When samples are found to be similar at this stage, the examiner should proceed with other examinations available and practical to adequately address the chemical, compositional and physical properties of the tapes before rendering a conclusion. At that point if no significant differences are found, the tapes are consistent and could have come from the same source. Only in rare circumstances can a stronger statement be supported.

Instrumental analyses of tapes have some limitations including the inability to detect elements in trace concentrations, the need for a conductive coating of the sample (with a high vacuum SEM), and the discoloration of materials by irradiation. Although quantitative and semi-quantitative methods are available for energy dispersive X-ray spectroscopy, they are not appropriate for most tape analysis because of the typical condition of the tape.

The information available from a heterogeneous specimen may diminish as its size is reduced and its condition degrades. The smaller a specimen, the less valuable it may become for an association. As sample size is reduced, it may no longer be representative of the original material. This may also be true of a degraded specimen.

Infrared spectroscopy can provide molecular information regarding major organic and inorganic components. For various reasons, components in lesser amounts are typically more difficult to identify unequivocally. Reasons for this include interference of the absorption bands of the major components with the less intense bands of minor constituents and sensitivity issues whereby the minor components are present at concentrations below the detection limits of the instrument. IR can be used to obtain spectra for elucidation of the chemical composition of a tape and for comparison of two or more samples. When used for comparison of spectra, the goal is to determine whether any significant differences exist between the samples.

The SEM is one component of the analytical scheme of the forensic analysis of tape and can be used to define the bulk elemental composition of individual tape components (backing and adhesive) and the elemental composition of individual particulate components within tapes, as well as the surface morphology.

Py-GC/MS can provide valuable organic chemical information of tape samples. The organic constituents of any tape are the polymer, elastomer, plasticizers, tackifying resins, and/or additives. These constituents may appear in the pyrogram and have comparative value. As pyrolysis techniques are destructive, the amount of sample available must be taken into consideration. This analysis is often placed at the end of an analytical scheme in which the combination of previous analytical techniques was incapable of discriminating samples. Since it may be able to add additional information that allows for discrimination between samples, its use is recommended for tape analysis and comparisons when sufficient sample is available.

Py-GC/MS is applicable to various polymer types. The pyrograms generated from Py-GC/MS can be used to compare the organic content of samples to identify most of the major organic constituents in polymer samples, enabling classification. This entails analyzing reference standards and empirically assigning peaks in the pyrogram. When used for comparison, the goal is to determine whether any significant differences exist between the samples.

Py-GC coupled with mass spectrometry is a very powerful and sensitive analytical technique that can be used to effectively characterize tape samples. This technique also provides information about the individual pyrolysis components of the pyrolyzate, which enhances the ability to chemically classify the different tape components. This entails analyzing reference standards and empirically assigning peaks in the pyrogram. The pyrolyzates produced are often not the same materials that were originally added in the manufacturing process prior to polymerization but frequently indicate the original materials.

37.3 **APPARATUS AND EQUIPMENT**

Standard laboratory equipment and a variety of solvents are required for the analysis of tapes and other synthetic polymers.

37.4 **PROCEDURE**

37.4.1 CASE APPROACH

The forensic examination of pressure sensitive tape encompasses the determination of physical construction and chemical composition of tape products. Methods for the analysis of tape include examinations of physical characteristics, polarized light microscopy (PLM), Fourier transform infrared spectroscopy (FTIR), pyrolysis gas chromatography-mass spectrometry (pyGC-MS), scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) and X-ray fluorescence spectrometry (XRF). These different procedures provide complementary information and should be selected and employed in an order to obtain the most discriminating information consistent with the laboratory's capabilities.

Typically, a tape examination involves the comparison of samples to determine if they could share a common origin. The goal is to determine if any significant differences exist between the samples. The evaluation of tapes for class characteristics can associate known and questioned tapes to a group but not to a single, individual source. A physical end match of two tape ends provides individualizing characteristics that associate the two tapes to one another to the exclusion of all other tapes.

Questioned tape samples may be submitted with a request to identify possible product information, manufacturing, and retailing sources. Sourcing of a questioned tape can provide valuable investigative lead information. Physical characteristics and compositional data are useful for technical inquiries to tape manufacturing companies, comparisons with various brands of tape purchased at local commercial outlets, and for searching reference databases.

If another discipline is chosen before the tape examination, obtaining an unadulterated representative sample should be considered. In some circumstances, it may be desirable to obtain a sample cutting from the tape before a sample is analyzed for latent fingerprints. Necessary precautions should be taken to eliminate loss or contamination of other evidence (e.g., latent prints, DNA, and other trace evidence).

When the amount of a tape specimen present for comparison purposes is adequate in size, bulk or lot sampling is the sampling method of choice. Considerations involved with bulk sampling should include where the sample is taken, how much sample is taken, and if the sample is considered representative of the whole. The examiner must be able to explain how the samples were taken and why the sampling technique was used. Nondestructive methods should be exhausted before subjecting the sample to any destructive tests.

If tape is received in a tangled condition, an attempt should be made to separate it manually with a careful peel. More aggressive techniques such as gentle heat, liquid nitrogen, freezing, or solvents can be used if necessary. However, these techniques could affect the outcome of subsequent analyses and should, therefore, be applied only to the extent necessary.

The item of evidence should be preserved in a manner that does not interfere with future testing.

Tape samples submitted as evidence may be degraded by environmental exposure or subjected to physical damage. The strength of an association between a damaged piece of tape and a more pristine sample might be weakened depending upon the degree of damage. In some cases, the damaged tape may be unsuitable for comparison purposes.

37.4.2 ANALYSIS

The following sections provide an overview of a suggested analytical scheme to be utilized for the analysis of tape. The selection of techniques is at the discretion of the examiner on a case-by-case basis and will vary depending upon the sample size or condition.

Physical Characteristics
Written descriptions, sketches, photography, or other imaging methods must be used to document each sample's characteristics.

A preliminary visual examination of tape construction should include its general appearance, both with the unaided eye and using a stereomicroscope.

For all pressure sensitive tapes, document and record any physical damage (e.g., worn, cut, torn, frayed). The following general visual characteristics should be observed and documented:

- General condition, including any adhering material
- Tape core markings and packaging information, if available
- Wads, flat pieces, or fragments
- Dimensions (e.g., width and length)
- Number of pieces
- Colors
- Condition of the ends for possible physical matches

Physical End Match: When conducting comparison examinations between two more tape specimens, the free ends should be carefully examined for possible physical end matches. Even though this type of association is the most compelling type of association, the analyst may elect to continue with a complete analytical analysis of these specimens depending upon the quality of the end match.

General guidelines for physical end match examinations:

- Observe the tear or cut pattern from the backing and adhesive side of both specimens to determine if a physical association is plausible. To observe finer detail, a stereomicroscope should be used to examine the ends.
- If the backing is distorted or folded over and adhered to the adhesive layer, gently straighten it out to restore the torn/cut edge. This may be accomplished with the careful use of forceps, gentle heat, mild solvent, or by freezing.
- Depending on the type of tape, manufacturing marks, creping on the paper backing, printing or any other continuous surface features may be present across fractured edges and would provide additional points of comparison.
- Determine if there are individualizing characteristics (e.g., a flaw or mark) that extends across the fracture. This would be an accidental or anomalous mark that initiates on one piece and terminates across the fracture edge on the other.

- If the tape has a fabric reinforcement layer, solvent (e.g., hexane, chloroform, or xylene) may be used to remove a sufficient amount of adhesive to expose the fabric and ensure alignment of the yarns that have broken across the torn ends.
- Any physical associations must be documented with descriptive notes. Physical associations between specimens that link a suspect to a crime scene or to a victim should be imaged. The imaging method should be dimensionally accurate and include a measuring scale if possible.
- It is required that all physical end match associations between a questioned specimen and a known specimen be verified by another qualified analyst. Refer to the Physical Match chapter regarding Quality Assurance.

Tape examinations involve a process of documenting all the physical characteristics exhibited. The following characteristics should be documented when applicable:

- Color of adhesive and backing
- Surface texture
- Width measurement
- Overall thickness
- Backing thickness

Physical Features: Each of these characteristics can have a number of sub-elements, all of which can be characterized to complete the examination. Physical characteristics of tape may change after removal from the original roll (e.g., weathering, sample handing). The analyst must decide what is an acceptable variation based on the circumstances of the case. Any measuring devices used should be properly checked with applicable quality assurance and control procedures.

Backing: The type backing must be recorded (e.g., paper, polymer film). The backing should be visually and stereomicroscopically examined for color, texture, and appearance under multiple illumination sources. For comparative examinations, a side-by-side color comparison of two or more backings is appropriate; otherwise, the Munsell or International Commission on Illumination (CIE) color systems may be utilized.

Markings on the backing: Using a stereomicroscope, the tape should be examined for features such as calendaring marks, striations, dimples, and inclusions. The shapes and type of markings should be documented.

Multiple layer backings: Tape backing should be examined to determine if multiple layers are present. This can be accomplished by cross-sectioning the tape backing via hand-sectioning or microtoming. One hand-sectioning method is as follows:

- The backing can be removed from the tape adhesive and fabric (though this is not necessary, particularly if the adhesive layer structure is also of interest).
- Two glass slides act as a sample holder by placing the bulk of the tape backing flat between them, with a small portion of the backing remaining outside the edges of the slides.
- The glass slides with the tape backing are attached to a holder (e.g., held with office tape to the sides of a 2” pillbox) and positioned under a stereomicroscope such that the slides and backing are perpendicular to the microscope platform.
- Liquid nitrogen or propellant from an aerosol duster is used to freeze that small portion of the backing to make it more rigid for cutting.
- A series of cuts are taken through the edge of the tape backing with a single-edged razor blade positioned nearly parallel to the platform and nearly perpendicular to the backing and slides. The razor blade should also be cooled along with the backing for efficient cutting. Very thin cross-sections are required for proper examination.
- The cross-section(s) are collected and examined with a compound microscope using transmitted light in order to determine layer structure.

The multiple layers should be characterized and then analyzed with appropriate analytical instrumentation.

Adhesive: The adhesive should be visually and stereomicroscopically examined for color and appearance under multiple illumination sources. For comparative examinations, a side-by-side color comparison of two or more adhesives is appropriate; otherwise, the Munsell or CIE color systems may be utilized. Some duct tape adhesives may be multi-layered, and cross-sections of the adhesives should be made when deemed necessary. The layer structure of the adhesive could be evaluated by examination of a cross-section of the *intact* tape, prepared using a method described in the multiple layer backings section above.

Reinforcement: If reinforcement, such as scrim or filament fibers, is present in a tape, it should be characterized.

Duct tape reinforcement: The three main features to examine in duct tape reinforcement are weave, yarn description and scrim count.
The weave of scrim fabric should be assessed using the stereomicroscope. This may require separating the adhesive from the scrim. The most frequently encountered weave patterns are weft-insertion and plain weave. Weft-insertion has chain-stitch warp yarns with textured filaments in the fill direction. A plain weave has a one over/one under pattern; the warp and fill directions can be a combination of any of the following types of yarns:

- Twisted yarns (Z- or S-twist)
- Filament fibers bound by another filament fiber
- Texturized filament fibers
- Straight filament fibers.

The fluorescence of the yarns/fibers should be examined.

The scrim count, the warp count per inch and the fill count per inch, should be measured and recorded.

Strapping (filament) tape reinforcement: The fibers in filament tape most often consist of synthetic or glass fibers. The fibers are only in the warp direction. The number of bundles across the width of the tape or per unit length should be counted.
The fluorescence of the filament fibers should be examined.

Within-roll variability of physical features: Within-roll variability in some measured physical features is possible, such as tape width, thickness, and scrim count. When variances are observed in the comparison of two tape samples in which all other features are similar, the analyst must decide on an acceptable tolerance. When available, within-roll variances are best derived from a known roll submitted with the case. Alternatively, similar products may be assessed to gain insight into the expected variances.

Polarized Light Microscopy

There is variability in tape films, adhesives, and fibers that can be readily noted with transmitted and polarized light. If differences can be seen by this technique, further tests are not necessary.

Other initial examinations, such as physical match, macroscopic/stereomicroscopic examinations, and the collection of trace evidence such as hair and fibers adhering to the tape, should be completed before proceeding with the sample mounting for the microscopic examinations.

Tape samples should be examined first under a stereomicroscope. Areas of the tape that appear in their original state (e.g., not stretched out of shape and having a clean adhesive area) should be selected for analysis. Fingerprint powders or chemicals should be gently but thoroughly cleaned from the film backing.

Fabric-Reinforced Tape (Duct Tape, Gaffer's Tape, Strapping Tape): All three layers of fabric-reinforced tape may be mounted separately for microscopy. The adhesive and reinforcement fibers will have more discriminating microscopic features than the film backing.

Sample Preparation: Areas of tape free of contamination are selected for analysis. This is best done under a stereomicroscope. Ends should be avoided when cutting samples. The tape is initialed at the site of the cut. The film backing is separated from the adhesive and fabric. A suitable solvent (e.g., hexane) may be used if mechanical separation is not feasible. The clean film is mounted in the appropriate medium and cover-slipped.

Microscopic examinations of the adhesive are useful only in opaque adhesives. The adhesive is separated from backing by pinching with tweezers and cutting with a scalpel and then transferred to a microscope slide. Care should be taken not to include fibers in this sample. Xylene or a similar solvent may be added to the adhesive sample on the slide to disperse the adhesive's rubber base. After drying, the sample is mounted in a suitable mounting medium and cover-slipped. Most minerals of PSAs can be evaluated in mounting media having refractive indices of 1.66 and 1.55.

Fibers from the scrim fabric can be gently pulled and clipped from the adhesive for mounting. If necessary, the fibers can be rinsed of any adhering adhesive using hexane or another suitable solvent. The warp and fill yarns may be cotton/polyester blends. Therefore, the whole bundle should be loosely mounted on a microscope slide in a mounting medium. Warp and fill yarn fibers are mounted separately.

Microscopic Examination of the Mounted Film Backing: Tape backings with some transparency may be cleaned of adhesive and mounted in a mounting medium. In duct tapes, the gray color of polyethylene film backing is due to the presence of aluminum powder. Viewing mounted duct tape films under transmitted light on a comparison microscope may offer some comparative information about the density, size, and dispersion of the aluminum particles in tapes. Note that duct tape backings may be multilayered. A cross section of the duct tape backing should be examined for physical characteristics and chemical composition. In clear and matte backings from strapping tapes and office tapes, additives are looked for and noted in plane polarized and cross polarized light.

Microscopic Examination of the Mounted Adhesives: The inorganic fillers of PSAs may be examined under transmitted plane and crossed polarized light. Mounting media with refractive indices of 1.66 and 1.55 are suitable for most mineral types that may be found in PSAs. The morphological and optical features of the different inorganic fillers can be noted. These particles are mixed with the elastomer and tackifying resin and include, but are not limited to, kaolinite, calcite, dolomite, rutile, zincite, or talc. Dispersion of adhesive samples first in xylene allows for a better assessment of these fillers. The identity of these minerals may be surmised from their optical properties along with the IR spectra and elemental composition.

Microscopic Examination of the Fiber Reinforcement: Refer to the Fibers chapter of this manual for methods of determining the optical properties of the reinforcement fibers of the tape. Using these microscopic methods the following observations should be made separately for the warp and fill fibers:

- Fiber class – usually cotton or polyester
- Diameter – of each class of fibers.
- Delustrant – either absent, light, medium, or heavy
- Shape – may be round, polygonal, tri-lobal, etc.
- Blending – cotton may be blended with polyester

Non-Reinforced Tape –Examinations of Oriented Films (Clear Polypropylene Packing Tape): The methods described in this section are recommended for clear packing tapes; however, they are applicable to other non-reinforced tapes with clear backings. Transparent ½" office tapes and some strapping/filament tape may have oriented polymer backings.

The variability in the polymer films in clear packing tape is imparted during the manufacturing process. Controlled heating, cooling and stretching produce films with both amorphous and crystalline areas. Biaxially oriented polypropylene (BOPP) is stretched in two directions with crystalline bundles lining up along the two stretched directions. Monoaxially oriented polypropylene (MOPP) is stretched in one direction. The differences in these two types of packing tapes can be distinguished with polarized light microscopy. Within each of these subclasses of packing tapes, variances may also be noted in the extinction direction with respect to the machine direction of the tape. Differences in interference colors will reveal differences in tape film thickness.

The polypropylene film of packing tapes behaves as an optically biaxial crystal. There are two perpendicular refractive indices in the plane of the film. One runs roughly in the cross direction and the other in the machine direction. The third refractive index runs normal to the plane of the film.

Sample Preparation: Select about an inch of tape that has both machine edges and appears to be in its original state (e.g., has not been damaged by heat, stretching, or contamination). Stick this piece directly onto a clean microscope slide adhesive side down. An arrow noted on the mounted sample can help keep track of which direction is the machine direction.

There is no need to separate the adhesive from the film for the microscopic examination of clear packing tape. Brown packing tapes with clear film backings and colored adhesive must have the adhesive removed. The cleaned film may be mounted in an appropriate medium for microscopic examinations.

Determination of Polypropylene Film Orientation: The following polarized light observations presume that the microscope is optimally aligned and illuminated.

The surface of the clear packing tape sample is brought into focus in transmitted light at about a 100X magnification. The polars are crossed, and the extinction position is found. The stage is rotated just off extinction, and patterns are observed in the film. These patterns may be sharpened by refocusing and closing down the aperture diaphragm. A pattern of "X"s is seen in biaxially oriented tapes (BOPP) and sows the bi-directional stretching in the production process. The pattern seen in monoaxially oriented tapes (MOPP) shows the one direction of stretching. Its pattern may be hazy and show more than one interference color that streak in the one direction of the stretch.

The angles of the crosshatches in the BOPP tape pattern described above may vary from one tape film to another but will be consistent throughout a roll of tape. These angles can be determined with an appropriate eyepiece reticule.

Determination of the Extinction Angle Relative to the Machine Direction: The machine direction of the tape relative to the extinction direction may vary from 0 to 15 degrees between different tapes.

The surface of the tape is brought in focus in transmitted light. One of the machine edges of the tape is aligned with the vertical line of the eyepiece graticule. The stage position is noted in degrees. The polars are crossed and the stage is rotated until the tape film is at is nearest full extinction. The stage position is again noted. The difference in degrees is the extinction angle relative to the machine edge. Tape samples from the same roll will show similar extinction angles.

Determination of the Retardation: The thickness of the polymer film in clear packing tapes can vary within manufacturers. When the birefringence of the films of the different packing tapes is the same, the variance in the interference color of the films will be a function of the thickness. Slight differences in thickness will show noticeably different interference colors. These interference colors depend only on the tape film thickness, not the total tape thickness (film + adhesive). The clear adhesive layer is isotropic and does not contribute to the interference colors.

Using approximately 400X magnification, the surface of the tape is brought into focus and the polars are crossed. The interference color is noted with the stage rotated to maximum brightness (close to 45 degrees). The fast wave (lower refractive index) is found with one refractive index running roughly across the tape and the other running roughly lengthwise along the tape. The fast wave is aligned parallel to the slow wave of a quartz wedge or Berek compensator. The point of compensation is found and from this, the retardation can be calculated. The experience of the microscopist may dictate the easiest way to arrive at the retardation value.

The birefringence of the polypropylene tape film (BOPP) in the plane of the tape (the difference between the refractive indices of the machine and cross directions of the tape) has been reported in the range of 0.014 – 0.016.

Other Observation: Some clear tape films may have additives that may be visible in transmitted and polarized light and their presence is useful for comparison purposes between tapes. Some assessment of their optical properties should be noted: size, distribution, relative interference colors, etc.

Irregularities in the thickness of the tape film may be observed under crossed polars as multiple interference colors in any given field.

Some tape films may not totally extinguish, or they may show undulose extinction (i.e., areas of lightness and darkness).

Microscopy of Other Tape Classes: Any tape class than has inorganic fillers in the adhesive or backing, reinforcement fibers, or clear or semi-opaque film backings may lend itself to examinations described in this procedure.

<u>*Infrared Spectroscopy*</u>

When analyzing difficult samples (e.g., residue, dirty samples, or inhomogeneous samples), care must be taken when sampling the tape and in choosing appropriate analytical conditions. An attempt should be made to remove any extraneous material from the specimen before sampling. In order to ensure reproducibility and/or evaluate intra-sample variations, repeat analysis of any samples is recommended.

Tackifiers and/or plasticizers may be extracted from adhesive or backing using a mild solvent such as hexane or acetone. They are subsequently analyzed in transmission by casting a thin film.

Transmission: Samples prepared for analysis by transmission techniques must be thin enough to allow infrared radiation to pass through without being over-absorbed by the sample.

Samples preparation techniques that may be employed for transmission analysis in the main bench include backing and/or adhesive pressed in a diamond cell, a thin backing sample stretched over an aperture, or adhesive deposited onto an alkali halide pellet (e.g., KBr, NaCl, or AgCl).

ATR: ATR methods may lend themselves to conducting the examination of the tape intact. Since ATR is a surface technique it is necessary to remove any extraction material from the area to be examined. The bench ATR (single reflection) is useful for forensic casework size samples. These accessories utilize an internal reflection crystal to condense the beam onto a spot-sized sampling area.

ATR is also useful in the analysis of duct tape backings for layer structure determination. The adhesive is removed, and the backing is analyzed on both sides. The compositions are then compared.

FTIR Microscope Accessory: The use of a microscope accessory is preferred for very small samples. Spectra can be obtained from samples as small as 10-20 micrometers in diameter after flattening.

There is a tradeoff between sensitivity and spectral range with MCT detectors. The low energy cut off for most detectors is in the 700-450 cm^{-1} range. The smallest apertures particularly limit the energy from the longer wavelengths (smaller wavenumbers) reaching the detector due to diffraction. Heterogeneity issues are also more pronounced when using very small apertures.

The microscope attachment permits the analysis of multiple samples placed on an appropriate support material. The method affords the advantage of viewing the sample optically and choosing the most appropriate area for analysis.

Spectral measurements using an FTIR microscope can be obtained in transmission, reflection, or ATR mode.

Transmission: Transmission measurements are commonly used because they generate spectra with fewer artifacts. However, transmission methods generally entail more sample preparation than reflection techniques. The tape sample must be rendered thin enough not to over-absorb. Samples can be placed directly over a small aperture for analysis or placed on an appropriate salt plate. This typically requires a sample thickness of approximately 3-5 micrometers.

A diamond cell can also be used as a sample support medium under the FTIR microscope. The adhesive can simply be smeared on one of the diamond faces. The tape backing sample is placed on one of the diamond faces, the second diamond is positioned on top, and sufficient pressure is applied to form a film. For nonelastic samples, one diamond is typically removed prior to analysis once the sample has been compressed. This leaves the think compressed film adhering to one of the diamond faces.

Reflection: If samples are flattened directly on an infrared light reflecting surface (e.g., low e-glass or gold mirror), the reflection mode can be used to produce spectra mimicking double-pass transmission spectra. The technique is sometimes referred to as “transflection” or “reflection/absorption.” Samples need to be approximately half the thickness of an optimum transmission sample.

The FTIR microscope can also be used in the specular reflection mode; however, it is not useful for tape unless the surface of the sample is highly reflective.

ATR: ATR objectives are available for infrared microscopes. Consistent pressure should be applied to each sample to mitigate spectral variations. Intra-sample variations may result from sample heterogeneity; therefore; multiple samplings should be considered.

<u>Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy</u>

Samples should first be examined with a stereomicroscope, noting size, color, structure, and any extraneous material adhering to the sample. The choice of a specific method for sample preparation depends on the size, nature, and condition of the specimen, as well as the analytical objective. It may be necessary to use multiple preparation methods in order to analyze all sample characteristics. In developing a strategy for analysis, the following should be considered:

- Determination of the presence of extraneous materials and a strategy for removal
- Method of attachment to a SEM mount.
- Method(s) for producing a uniform geometry
- The need for a conduction coating on the prepared samples
- Determination of the presence of surface features of analytical interest

If the analytical objective is to determine elemental composition, then any possible contribution from extraneous materials should be eliminated or accounted for. For accurate comparison of elemental composition and structure, samples should be prepared in the same manner.

Recognition and removal of extraneous materials: It is not unusual for extraneous materials to be present on the surface of a specimen submitted for analysis, particularly on an adhesive component. Because the SEM method is a surface analysis, the presence of even a small amount of this material can prevent an accurate determination and comparison of composition. Therefore, recognition and removal or abatement of this material should be performed.

Depending on the sample size and type, extraneous material may be physically removed with a brush, probe, or fine blade. Debris can also be removed from the backing surface with methanol and a cotton swab or lifted off the backing with office tape. Care should be taken that the adhesive from the office tape does not adhere to the sample surface, which might interfere with any subsequent organic or inorganic analysis. If necessary, a fresh surface may be exposed by scraping or cutting with a fine scalpel blade.

When extraneous material cannot be removed, these materials should be avoided during analysis.

Methods of attaching samples to SEM mounts: All samples to be analyzed in the SEM should be attached to an appropriate SEM mount. Because the presence of a carbon peak in the spectrum does not usually interfere with elemental comparisons, mounts constructed of carbon are preferred.

The adhesive should be removed from the backing to ensure no contribution from the backing is in the resulting spectrum. The tape's adhesive is smeared directly onto the surface of the mount.

A backing may be attached directly to a mount using the tape's own adhesive. Contribution of the adhesive in the resulting spectrum is typically not a concern during backing analysis. If the backing has been separated from the adhesive or if a cross-section of the backing has been prepared, the backing or cross-section can be attached to the mount with a mounting adhesive. This mounting adhesive may be applied as a liquid or as a double-sided tape. If the tape is known to be multi-layered, both the outer and the adhesive side may be analyzed.

The geometry of each sample, including flatness and take-off angle, should be similar. Often, a backing can be pressed flat with clean glass in order to remove irregularities.

Generally it is necessary to apply a conductive layer to the sample surface to eliminate charging. Carbon is preferred, because the presence of a carbon peak in the spectrum usually does not interfere with elemental compositions. The use of a variable pressure instrument may also eliminate charging.

Structural imaging: SEM imaging of pressure-sensitive tape backings at moderate magnification (75-250X) yields structural information complementary to that of traditional light microscopic methods. It can be used to image very small striations, craters, and surface features on the backings of polymer-based tapes, such as black electrical tape and duct tape. It can be used to view the paper fibers in masking tapes, as well as show the cross-sectional structure of each of these tapes.

A backscattered electron image is useful for defining structures based on the average atomic number of the matrix. Structures containing elements with higher atomic numbers will generally appear brighter than those with lower atomic numbers. This is often useful for evaluating homogeneity and layer structure.

SEM micrographs should include a measuring scale or magnification scale or both. The micrograph should also display which signal (backscattered electron or secondary electron) was used to produce the image.

Bulk spectra collection: Once heterogeneity of the material is evaluated, a spectrum of the average (bulk) elemental composition of the sample is obtained. The raster should include as much area of the sample as possible. Analyzing a single large area or summing the spectra from several smaller areas may achieve this.

Qualitative analysis: Once an X-ray spectrum is collected, a qualitative analysis is performed in order to determine the elements present. The process is straightforward for the peaks of elements present in major amounts and those not overlapping. Misidentifications or omissions of minor components are possible unless a systematic approach to elemental identification us used which includes consideration of X-ray families, spectral artifacts, escape peaks, sum peaks, and overlaps.

Individual component analysis: Additional evaluation of composition may be achieved by the spot (nonrastered) analysis of specific particles within layers. Generally these particles appear bright in the backscattered electron image. Such an analysis may improve the detection by a bulk analysis. For example, the bulk analysis of a tape adhesive may reveal the presence of Al, Si, MG, and O. Specific particle analysis may associate the elements Si, Mg, and O as being present in one type of particle, and Al, Si, and O in a second type. These associated elemental compositions would then indicate these particles could be talc and kaolinite, respectively. Polarized light microscopy, infrared spectroscopy, or X-ray diffractometry can be used to confirm the presence of some of the compounds.

Because beam interaction volume may be considerably larger than an individual particle, inclusion of other matrix components may be expected in the spectrum from an individual particle. Lower beam voltages may be used to confirm more of the interaction volume to the particle. It should be noted, however, that the use of lower beam voltages may result in the loss of characteristic lines that may be found at higher energies.

Analysis of a primary organic matrix: Analysis of a substance that is primarily organic (e.g., duct tape backing, clear electrical tape adhesive) may be useful. Within such a matrix, the interaction volume is significantly larger than that of a substance that is primarily inorganic. This is a result of a lower average atomic number of the matrix. In order to reduce the interaction volume, the beam voltage may be reduced; however, the voltage should be sufficient to produce X-rays from all lines of analytical interest. Charging may also be an issue with such samples. Therefore; precautions may be taken to prevent this from occurring (e.g., sample coating or operation at low vacuum).

Because an organic matrix may contain small amounts of some elements, the counting time should be extended.

Heterogeneity versus analytical area: In order to compare the average composition of structures, the spectrum used for comparison should come from an area of the structure to produce representative composition.

The representative nature of a spectrum can be determined by the critical comparison of spectra from adjacent areas. If not differences are evident, the sampled area is homogeneous at that magnification. A representative bulk analysis can be achieved by rastering the beam across as large an area as the sample permits.

Pyrolysis-Gas Chromatography/Mass Spectrometry

The sample to be analyzed should first be examined with a stereomicroscope to ensure that the sample is free from any foreign material. Sample preparation should be carried out using a stereomicroscope, and clean tools must be used to handle the sample and the quart tube or platinum foil. Samples to be compared should be prepared in the same manner resulting in approximately equivalent sizes and should be analyzed using identical instrument conditions.

Sample size is typically on the order of 10 to 150 micrograms, depending on instrument sensitivity and chemical composition of the material (e.g., amount of inorganic filler, type of elastomer), and should be approximately equivalent for all samples to be compared.

Removing the adhesive from a substrate for analysis can be done by rolling a metal probe along the tape, allowing the adhesive to collect on the probe. Alternatively, a scalpel can be used to tease up some of the adhesive. The collected adhesive is then transferred to the pyrolyzer sampling device using a scalpel, tweezers, or other suitable tool. This tool should be wiped clean with acetone or other suitable solvent between uses.

The backing can be analyzed separately. It can be sampled by removing the adhesive using an appropriate solvent, or thin peels can be taken from intact tape.

Pyrolysis Temperature: Temperature control of the pyrolysis process enables reproducibility of the polymer fragmentation. The pyrolysis temperature must allow for its complete degradation without causing excessive bond breakage. Too much fragmentation will render the resulting pyrogram very difficult to interpret. The pyrolysis unit must pyrolyze the sample at a set temperature and/or at a reproducible heating rate for a specific duration.

Gas Chromatograph Parameters: The GC must have a reproducible temperature profile and a stable carrier gas flow rate. Column type, gas-flow rates, and temperature programs influence the pyrograms obtained during analysis. The conditions should be chosen based on the quality of pyrograms they produce with regard to peak resolution and repeatability.

Mass Spectral Range: A scan range should be chosen in order to allow analysis of breakdown products of potentially large molecules (e.g., polymers) while disregarding lower weight fragments that may unnecessarily clutter the mass spectrum. Usually the mas range starts between 30 and 50 mass units and ends about 500 to 650 mass units.

Example Experimental Conditions: Instrument parameters will vary depending on the instrument system. The following parameters may be used as a starting point for the Py-GC/MS analysis, but each laboratory should establish its own optimized parameters.

- Pyrolysis temperature and time: 700°C for 10 sec.
- GC oven temperature program:
    - Interface temperature: 275°C
    - Column: Non-polar capillary column (30 m x 0.25 mm ID)
    - Carrier gas: Helium
    - Pressure: 200 kPa
    - Split flow ratio: 75:1
    - Oven program: Column remains at 40°C for 2 minutes
Ramp temperature 6°C/min to 295°C
Hold at 290°C for 5 min.
Total run time: ~47 minutes
- Mass spectrometer:
    - Scan speed: Scanned 1000 m/z per sec
    - Time interval: 0.5 seconds
    - Mass range: m/z 50-500
    - Transfer line: 290°C

Sample Introduction: Quartz tubes or other sample introduction containers/methods (specific to different types of pyrolysis units) may be used depending on the instrumentation.

Blanks: A system blank should be run prior to analyzing each sample (evidentiary and reference standard) to ensure and demonstrate that there is no contamination and/or carryover. The system blank should include all aspects of the system, including the sample container. Cleaning or bake-out blanks can be used after samples and prior to subsequent system blanks.

Performance Check: Prior to use, the performance of the instrument must be verified. Refer to the GC/MS section of this manual.

37.4.3 NOTE TAKING

Tape may not be in its original state due to weathering, stretching, chemicals, etc. These changes may limit the information obtained from the analysis. If the tape does not allow for the full range of examinations, the examinations and analyses that are performed should be reflected in the analyst's notes, and the reasons for the limited examinations should be documented.

The goal is to produce documentation that will be meaningful to a reviewer in the absence of the recording analyst. The resulting notes must be sufficient to support the conclusions reached in the analyst's report. All pertinent data, including any documentation of physical end matches, should be placed into or referenced with the case file.

When making comparisons of tape samples, similarity or dissimilarity of tape samples should be noted.

37.4.4 PACKAGING

Package accompanying trace evidence in appropriate containers

Secure tape evidence and the tape ends used in physical matches so that the three dimensional details are retained. Mark the tape ends for future reference.

37.5 **INTERPRETATION AND REPORTING**

For comparative tape examinations, if significant differences are observed in physical characteristics, no further testing is necessary, and a report can be issued. If no significant differences are observed, instrumental examinations should be performed before a report is issued. Any limitations that affect the conclusions (e.g., sample size, condition of the sample) should be addressed in the report. In sourcing cases, instrumental examinations may be necessary before a report is issued.

37.5.1 IR SPECTRAL INTERPRETATION:

Comparisons of the tape component spectra can be accomplished by digital overlays with full scale expansion. Comparison of samples may be conducted with both spectra displayed in transmittance and/or absorbance. Certain information may be seen more readily in one format or the other.

There are a number of significant factors that should be considered when comparing spectra including the presence or absence of absorption bands, and their position (wavenumber), shape and relative intensity. Additional sample replicates may be necessary to evaluate reproducibility of these spectral characteristics:

- The presence of additional absorption bands could be from true differences between the samples or from extraneous material adhering to the tape. If extraneous material is suspected as the source of the difference, the sample should be cleaned or additional samples prepared. If the sample cannot be cleaned or resampled, the spectral subtraction may be an option.
- For spectra to be considered indistinguishable, the position of the absorption bands should have reasonable agreement with each other. A rule of thumb is that the positions of the corresponding peaks in two or more spectra being compared should be within a few wavenumbers of each other, depending on whether the peak is sharp or broad. For sharp absorption peaks one may use tighter constraints and with broad peaks the variation may be slightly greater.

- For spectra to be considered indistinguishable, the shape of the absorption bands should be consistent between comparison samples. The peak width and the symmetry of each peak should be evaluated. Sample thickness may affect the peak width and resolution.
- For spectra to be considered indistinguishable, the relative intensities of respective absorption bands should be similar between comparison samples. The relative intensities may be affected by the heterogeneity of the sample.

Three possible conclusions can be reached after evaluating and comparing spectra:

- The spectra are dissimilar if there are one or more significant differences in the spectra. Significant differences are differences in which the spectral variation cannot be explained other than as differences between samples.
- The spectra are indistinguishable when there are no significant differences in the spectra. Differences are not significant if the spectral variation can be explained as something other than differences between samples.
- An inconclusive determination is one in which the significance of the differences cannot be completely assessed due to the constraints of sample size and/or condition.

37.5.2 COMPONENT CHARACTERIZATION:

Tape is often comprised of a number of components that result in overlapping bands in the IR spectra; therefore, caution must be exercised when evaluating the data. Not all of the components of tape can be elucidated by IR due to overlapping bands and/or relative concentration.

Tools that can assist in the characterization of the spectra include, but are not limited to, spectra libraries, flow charts, and reference standards. It should be noted that most commercial spectral libraries consist of transmission (as opposed to reflection) spectra. It is desirable to use reference spectra that were obtained using the same sample preparation and collection technique.

The following components, if present, may be characterized by IR spectroscopy depending on the condition of the tape and on the concentration of the material:

- Backing
    - Polymer film
    - Plasticizers
    - Fillers/extenders
    - Flame retardants
- Adhesive
    - Elastomer
    - Tackifiers
    - Fillers/Extenders
- Release coating
- Fiber reinforcement

37.5.3 ASSESSMENT OF SEM-EDS RESULTS:

Generally, comparisons are facilitated by direct spectral comparison. If spectral differences are not detected, it is likely that the materials are similar in elemental composition. If spectral differences are detected, it is likely that the materials are not similar composition; however, several alternative explanations are possible and should be evaluated.

- Differences in background shape may result from dissimilar sample geometry.
- Differences in the composition of major peaks may indicate that the spectra are not representative of the bulk composition of a heterogeneous sample. This could occur as a result of the analysis of a sample too small to be representative or the analysis of a raster area too small to be representative.
- If there are no differences in major peak ratios, differences in minor/trace components may results from the presence of extraneous materials. If the samples was a fragment or unable to be cleaned, a small amount of foreign material may have been present during the analysis.

Consequently some of the minor elemental peaks in the spectrum may have been produced from elements in the extraneous material.

- Differences in carbon intensity may result from a contribution of carbon from the mount if the sample is very small. Furthermore, the presence of carbon, oxygen, and nitrogen in the tape matrix limits the usefulness of these elements in direct spectral comparison; therefore, they are not typically evaluated.

37.5.4 INTERPRETATION OF SEM-EDS DATA:

A conclusion regarding similarity results from the comparison of images and elemental composition of individual layers. Spectra may be critically compared by overlaying them. If a comparative analysis did not demonstrate significant differences, then no differences were indicated in structure and composition within the limits of the analytical capability of SEM-EDS.

If ratio differences between peaks exist, it can be concluded that these differences may result from either actual differences in the bulk composition of the materials or from the analysis of a small sample (or area) whose chemistry is not representative of the bulk composition of a heterogeneous sample. The latter should be concluded following an extensive investigation of the heterogeneity of the known samples and demonstration that the range of variation present in one sample encompasses that observed in the other sample.

If there are no differences in major peak ratios but there are differences in minor/trace peaks it can be concluded that no differences in major elemental constituents are indicated, although some differences in the bulk composition are evident. For example, if the specimen was a fragment and unable to be adequately cleaned, a small amount of foreign material may have been present during the analysis. Consequently, some of the minor elemental peaks present in the spectrum may have been produced from elements in the foreign material and not from elements in the questioned material. Equally so, the observed differences may be due to actual differences in the composition of the samples. Therefore, with respect to the elemental composition of these samples, an inconclusive result for this technique is indicated.

If a comparative analysis demonstrates significant differences between samples regarding structure and composition, it then can be concluded that the samples are different.

37.5.5 INTERPRETATION OF PY-GC/MS DATA:

Pyrolysis techniques are suitable for the identification of polymers by their pyrolysis products at specified measurement conditions. Combined with retention times, pattern of chromatographic peaks and mass spectra of pyrolysis products can be used to compare and to identify pyrolysis products. Identification can be accomplished by comparison of a known sample, questioned samples, or both to a reference library or a contemporaneously analyzed reference sample. The library chromatograms should originate from the same instrument and protocol used in the current analysis. When possible, the standards used in creating the library should be traceable reference standards. When MS is employed, individual chromatographic peaks can also be identified via mass spectral library searches. The components identified may aid in determining the original starting materials of the manufacturing process.

Comparison of the pyrograms can be accomplished side-by-side or through overlays. There are a number of significant factors that should be considered when comparing pyrograms, including the presence or absence of peaks, retention times, shapes, and relative intensities. Additional sample replicates should be performed to evaluate reproducibility of these pyrogram characteristics. The presence of additional peaks could come from true differences between the samples or from extraneous material adhering to the sample. If extraneous material is suspected as the source of the difference, the sample should be cleaned and additional replicates analyzed.

For pyrograms to be considered indistinguishable the retention times of the peaks should have reasonable agreement with each other. Positions of corresponding peaks in two or more chromatograms being compared should be within 2% of each other. Additionally the retention time and the intensity and shape of the peaks should be consistent between comparison samples. The peak width and the symmetry of each peak should be evaluated. In practice, very polar substances, for instance, often form broad peaks when using a non-polar column; in this case, the reproducibility of retention time, peak intensity, and peak shape may be relatively poor. Sample size may also affect the peak width and resolution. For pyrograms to be considered indistinguishable, the relative intensities of the major respective peaks should be similar between comparison samples. The relative intensity may be affected by the heterogeneity and/or size of the sample. If replicate analyses are conducted, they could demonstrate a range of possible relative intensities

Three conclusions can be reached by evaluating and comparing pyrograms:

- The pyrograms are dissimilar if there is at least one significant, reproducible difference in the pyrograms. Significant differences are differences in the presence or absence of a peak or in relative peak intensities. These differences are too large to be explained by factors such as heterogeneity, contamination or poor reproducibility.
- The pyrograms are indistinguishable if there are not significant differences in the pyrograms. Differences are not significant if the variation can be explained by factors such as heterogeneity, contamination, or poor reproducibility.
- An inconclusive determination is reached if the significance of any possible difference(s) cannot be completely assessed (e.g., sample size constraints).

37.6 **QUALITY ASSURANCE**

Samples being compared should be prepared and analyzed in the same manner. Sample and background FTIR scans should be run under the same instrument conditions. When comparing samples by SEM-EDS, all data and micrographs should be collected in the same manner with the same conditions.

38 VEHICLE LAMP

38.1 INTRODUCTION

Forensic lamp examinations involve the assessment of vehicle lamp integrity to determine if a lamp was on or off during an accident. The examination process is based primarily on macroscopic and microscopic observations. Microscopic methods used to perform vehicle lamp examinations include stereomicroscopy and SEM. Some materials may be confirmed by elemental analysis with either SEM-EDX or µXRF.

38.2 ADVANTAGES AND LIMITATIONS

Vehicle lamp examinations are conducted by obtaining lamps from the area of impact on the vehicle and examining the filaments and other portions of the lamp affected by the filaments. Examinations of other lamps at a distance from impacts can only yield information as to whether the lamp is functional based on continuity of the filaments. These types of exams should be limited to lamps from motor vehicles (i.e. cars, trucks, motorcycles), since the empirical data upon which these lamp exams are based come from motor vehicles. If lamps from other types of vehicles are examined, caution must be used in extrapolating motor vehicle data.

Caution must be used in interpreting the observations because of the large number of variables that exist in most incidents. For this reason it is very important that an examiner obtain from the investigator an adequate history of the incident with photographs and crime scene sketches.

38.3 APPARATUS AND EQUIPMENT

An electric charcoal starter, a lamp testing apparatus, a multi-meter, an SEM-EDX and/or µXRF, and a variety of standard laboratory instruments and supplies are needed in the analysis of vehicle lamps.

38.4 PROCEDURE

38.4.1 CASE APPROACH

Prior to beginning a given case, contact the detective/agency to obtain pertinent case information, if necessary.

Lamp filaments are very delicate and easily break, therefore **ALL LAMPS SHALL BE HAND DELIVERED** to the laboratory. When receiving lamp evidence, check the evidence packaging for the presence of damage (i.e., tears, cuts, etc.), making a notation of any damage detected.

The examiner should make a conscious effort to maintain a separation of the samples, avoiding contamination at all steps of collection and examination.

For general details on techniques needed for sample recovery and handling, see the Trace Evidence Recovery chapter of this manual.

Obtain the following incident history, if available, in order to interpret observations:

- Which vehicles are the sources for which lamps? From which locations on the vehicle(s) did the lamps originate?
- What types of vehicles, or vehicle(s) and object(s) were involved in the impact?
- What velocities and/or accelerations were the vehicle(s) undergoing?
- How far from the impact site were the lamps?
- How much damage was done to the vehicle in an area within approximately 4 feet of the lamps?
- How many impacts occurred which affected the vehicles that the lamps came from? Which were the primary impacts?

- What were the lamp switches' positions after the incident? Did anyone change the position of the light switches after the incident occurred?
- Were there any problems in removing or packaging the lamps? Were they difficult to remove, dropped, found lying beside the vehicle?
- How soon after the incident were the lamps collected? If the vehicle was stored before lamps were removed, was it stored inside or outside and for how long?
- Obtain the year, make and model of vehicles involved in the accident(s).

38.4.2 <u>ANALYSIS</u>

- Label all lamps as to vehicle source (or item #) and location, if possible.
- Describe, sketch and photograph lamp or remnant, and condition. Include at a minimum the following information on the condition of the filament (s).
  - Luster and color
  - Shape and degree of deformation
  - Coil uniformity
  - Adhering traces such as glass and oxidation
  - Filament morphology at broken or melted ends
  - Using multi-meter check filament for continuity, if applicable
  - Sealed beam headlamps and outer glass envelopes of halogen headlamps, if intact, should normally be broken open for a complete examination
  - Filaments in bulbs can be examined through the glass envelope. If it is necessary for complete examination, break the glass envelope of non-halogen bulbs.
  - Tape broken glass edges with masking tape to protect against cuts.
  - If possible, in double filament lamps, determine which is high and low beam (headlamps) or which is the running light and signal/brake light (other lamps).
- Examine filaments macroscopically and with a stereomicroscope. An alternate light source may be used to better visualize films on glass due to the oxidation chemistry.
- Electrical continuity is determined by the use of a multi-meter.
- Record observations and any changes made to the lamp(s) during examination.

<u>Procedures for breaking open lamps</u>
Use methods described in the Vehicle Lamp Training Manual or any other that will not jar or alter filaments.

Glass headlamps

- If non-halogen, break nipple on back of lamp using a tool such as pliers. Listen for the hiss of gas intake to check for a vacuum indicating the lamp was intact.
- Heat the back of the lamp using an electric charcoal starter or equivalent point heat source and drip water on zone until glass breaks.

Plastic headlamps

- Use a saw on the back of the lamp
- Other glass lamps, non-halogen
- Slowly tighten a vise around glass envelope until the glass breaks. Protect the bulb during this process by wrapping bulb in some material that will catch glass and loose filaments. Suggestions for materials are plastic bag, Styrofoam cup, etc.

Halogen and High Intensity Diode (HID lamps

- Halogen and HID lamps are under extreme pressure, especially when incandescent. Extreme caution should be exercised when opening these types of lamps

<u>Stereobinocular microscopy</u>

The stereoscope is the primary tool used in the examination of lamps. You can examine all the exterior and interior components at a magnification typically between 4 and 100 times.

Scanning Electron Microscopy (SEM)
An SEM may be used when higher magnification and resolution is needed to observe and document annealed glass on the filament and filament damage such as brittle vs. ductile deformation.

Elemental Analysis
An SEM-EDX or µXRF may be used to determine the elemental composition of suspected glass particles and oxidation chemistry observed on the internal components of a lamp. The choice of which instrument is to be used will be based on the nature of the evidence as well as the advantages and disadvantages of each instrument.

38.4.3 NOTE TAKING

Follow the general note taking guidelines.

38.4.4 PACKAGING

Intact lamps should be packaged with soft packaging materials to protect lamps from jarring or coming in contact with each other. Sturdy outer packaging such as boxes is recommended.

Lamps broken open during laboratory examinations should be securely taped and packaged as in intact lamps. Broken lamp pieces should be packaged such that exposed posts and filaments do not come into contact with other parts. Rigid cups (Styrofoam works well) are good packaging material, but anything can be used that achieves the purpose.

All lamp examination evidence should be hand delivered to the laboratory to minimize shock and possible breakage which could occur during shipping.

38.5 **INTERPRETATION AND REPORTING**

The lamp examination report should include the lamp type, location and if possible function of each filament within the lamp. Lamp condition should be clearly stated detailing any and all abnormalities. The filament and glass envelope may be either broken or intact, so the initial classification falls into one of four possible categories:

- Glass envelope intact, filament broken
- Glass envelope intact, filament intact
- Glass envelope broken, filament broken
- Glass envelope broken, filament intact

Each type of failure can be further classified by the conditions which have given rise to the final state of the lamp:

- Mechanical shock when cold
- Mechanical shock when hot
- Glass envelope fractured while filament illuminated
- Lamp was switched on after the envelope fracture
- Slow air leak in envelope
- Normal filament burnout due to age
- Electrical short in filament
- Production

An indication of filament electrical continuity should be included in the report if warranted. Record an opinion as to what each abnormality signifies. Sometimes the conclusion may be that of indeterminate.

Such findings can occur when the lamp is destroyed or missing, or not enough filament is left to show signs of being on or off. Another indeterminate result will occur when the lamp appears normal with unbroken glass and impact shock was not enough to affect the filament. A degree of certainty of conclusions may be noted: possible, probable, and certain. Conclusions may be based on other things besides the lamp examination, such as damage to the vehicle, condition of the wiring, switches, fuses, and examination of other lamps and filaments associated with the specimen examined. Reports often state a lamp was incandescent (on/illuminated) when the glass envelope broke. It usually cannot be stated that the lamp was incandescent (on/illuminated) during the impact in question. This is due to the fact that the lamp could have broken in an incident prior to the one in question.

38.6 QUALITY ASSURANCE

An adequate set of reference books, articles, and manuals on vehicle lamps and lamp examinations is essential. New lamps obtained from a variety of sources and manufacturers are essential. Used and damaged lamps obtained from accidents and other sources are also important reference materials.

38.7 SAFETY

Broken glass can have sharp edges. Care should be taken when handling glass pieces. Glass fragments collected from clothing, and those with visible bodily fluids should be treated as potential biohazards. Halogen and HID bulbs are under high pressure and can present an explosion hazard if broken.

39 APPENDIX A: REAGENT PREPARATION

39.1 POTASSIUM THIOCYANATE CHEMICAL ENHANCEMENT

The thiocyanate ion, in an acid environment, will react with iron ions. Since iron is frequently found in soil and fertilizers, this method is a good choice for dirt or dust impressions.

39.1.1 FORMULA

Mix 15 ml of water with 120 ml of acetone

Add 15 g of potassium thiocyanate

Add 8.5 ml of dilute sulfuric acid (1 ml of concentrated sulfuric acid to 9 ml of water) to the above mixture

CAUTION: Always add the sulfuric acid to the acetone/water mixture. Do not add the acetone/water mixture to the acid or it may explode.

A milky mixture will result which will separate on standing. When the layers have separated, the top (clear) layer is removed and transferred to a glass bottle or spray unit. This is the working solution and is best if used immediately. If not used immediately, it is best to be stored in a dark glass bottle.

39.1.2 REAGENT CHECK

The reagent is checked by using ferric chloride (or a comparable iron standard). A positive reaction will result in a red/brown color.

39.1.3 APPLICATION

The impression, or area with suspected impression, should be photographed before application.

It is best to check the potassium thiocyanate solution with the material which makes up the impression. A portion of this material is removed (if possible) and sprayed. If there is only a small amount of material which makes up the impression (and removal could disturb the impression) then a portion of the impression is isolated by a physical barrier and sprayed. A positive reaction will result in a red/brown color.

If no positive reaction occurs, the potassium thiocyanate enhancement should cease.

The solution is lightly sprayed (fine mist) and the amount of spraying should be controlled to get the maximum reaction without causing the impression to run or bleed.

The impression should be photographed after application.

39.1.4 REFERENCE

Bodziak, *Footwear Impression Evidence*, 2000.

40.1 AMIDO BLACK – ONE STEP (WATER BASED)

This enhancement procedure uses a water soluble dye that reacts with the protein in blood that produces a dark blue-black color in areas where blood is present. This amido black water based formula is a one-step process which eliminates the need for a separate fix solution as it is incorporated into this formula. The amido black method can be used after treatment with leucocrystal violet (LCV) enhancement to further increase contrast.

40.1.1 FORMULA

Using a stirring device, combine the following ingredients in the order that they are listed.

500 mL	Distilled water
20 g	5-Sulfosalicylic acid
3 g	Amido black (also known as amido 10B or naphthalene black)
3 g	Sodium carbonate
50 mL	Formic acid
50 mL	Acetic acid
12.5 mL	Kodak Photo-Flo 600 solution

Dilute this mixture to one liter using distilled water. For best results allow the mixture to stand (if possible) for several days prior to use.

NFPA Rating (estimated): Health – 3, Flammability – 2, Reactivity – 0, Special – *none*.

40.1.2 REAGENT CHECK

Test the reagent with a known blood control. A positive reaction is a dark blue-black color.

40.1.3 APPLICATION

The impression, or area with suspected impression, should be photographed before application.

Using the amido black reagent, stain a small area of the evidence that is separate from the impression to check for background staining. If background staining occurs and will not rinse away with water, use a different enhancement method.

Apply the reagent to the area by either dipping, using a squirt bottle or apply using a fine mist. Completely cover the area in question and allow the area to develop for approximately 2 – 5 minutes. Once developed, rinse the area with distilled water.

Once enhanced, the impression(s) should be photographed.

Amido black can be used after treatment with LCV enhancement to further increase contrast.

40.1.4 REFERENCE

Bodziak, William J. *Footwear Impression Evidence: Detection, Recovery, and Examination*. 2nd Ed. Boca Raton, FL: CRC Press; 2000.

40.2 **AMIDO BLACK (METHANOL BASED)**

This enhancement procedure uses a water soluble dye that reacts with the protein in blood that produces a dark blue-black color in areas where blood is present. This amido black methanol based formula is a three-step process which requires the need for a separate fixative solution. The amido black method can be used after treatment with leucocrystal violet (LCV) enhancement to further increase contrast.

40.2.1 FORMULA

Fixative Solution:

20 g	5-Sulfosalicylic acid
1000 mL	Distilled water

Thoroughly dissolve the 5-sulfosalicylic acid in water.

Staining Solution:

900 mL	Methanol
100 mL	Glacial acetic acid
2 g	Amido black (also known as amido 10B or naphthalene black)

Thoroughly dissolve the amido black in the acid/methanol solution.

Rinsing Solution:

900 mL	Methanol
100 mL	Glacial acetic acid

40.2.2 REAGENT CHECK

Test the reagent solutions with a known blood control. A positive reaction is a dark blue-black color.

40.2.3 APPLICATION

The impression, or area with suspected impression, should be photographed before application.

Fix the impression with the Fixative Solution and rinse with distilled water. Stain a small area of the evidence (separate from the impression) to check for background staining. If background staining occurs and will not rinse away with water, use a different enhancement method.

Apply the staining reagent to the area by either dipping, using a squirt bottle or apply using a fine mist. Completely cover the area in question and allow the area to develop for approximately 2 – 5 minutes. Once developed, use the rinsing solution and allow the area to dry. This step should not be eliminated as it helps to remove the stain from the non-impressioned background area(s).

Once enhanced, the impression(s) should be photographed.

40.2.4 REFERENCE

Bodziak, William J. *Footwear Impression Evidence: Detection, Recovery, and Examination*. 2nd Ed. Boca Raton, FL: CRC Press; 2000.

40.3 LEUCOCRYSTAL VIOLET

Leucocrystal violet (LCV) is the reduced or colorless form of crystal violet. When LCV and hydrogen peroxide come into contact with hemoglobin or its derivatives, a violet colored dye (crystal violet) is formed through the catalyzed oxidation of peroxide. On contact with LCV, blood turns a dark violet color. This formulation includes a blood fixative, 5-sulfphosalicylic acid. Amido black can be used after LCV treatment to further increase contrast.

40.3.1 FORMULA

Dissolve 10 grams of 5-sulfosalicylic acid in 500 mL of 3% hydrogen peroxide using a 500 mL-bottle. (The 3% hydrogen peroxide sold in 473-mL bottles in stores also can be used.)

Add 3.7 grams of sodium acetate.

Add 1 gram of leucocrystal violet.

This solution must be stored in an amber bottle as it is light sensitive. This solution may be refrigerated to extend its reactivity. The solution shelf life is 30 days.

40.3.2 REAGENT CHECK

Note: if the LCV crystals are yellow instead of white, do not use! This means that the crystals are old and the solution may not be effective.

Test the reagent with a known blood control. A positive reaction is a dark violet color.

40.3.3 APPLICATION

The impression, or area with suspected impression, should be photographed before application.

Using the LCV reagent, spray a small area of the evidence that is separate from the impression to check for background staining. If background staining occurs and will not rinse away with water, use a different enhancement method.

Apply the reagent to the area by spraying a fine mist, soaking the area or by cascading the LCV over the area's surface.

On non-porous surfaces, such as tile, and on porous surfaces, when possible, the area should be rinsed with water approximately 2 to 3 minutes after the reagent has been applied.

Once enhanced, the impression(s) should be photographed. LCV luminesces and can be viewed and/or photographed under various wavelengths of ultraviolet and infrared light.

40.3.4 REFERENCE

Bevel, T and Gardner, *Bloodstain Pattern Analysis*, 2nd ed., CRC Press, Boca Raton, FL., 2002.

40.4 COMMON SEIZED DRUG COLOR SCREENING TESTS

The following are color tests commonly employed in the analysis of seized drugs. Many other color tests are well documented in literature and the following list is not intended to be all inclusive. The superscript number listed with the test name indicates the reference from the suggested reading list.

40.4.1 TABLE 1. ONE STEP COLOR SCREENING TESTS

Test Name	Reagent Formula	Positive Control	Expected Result
Cobalt Thiocyanate [1]	2% (w/v) sol'n of $Co(SCN)_2$ in water	Cocaine	Blue (flaky precipitate)
Froehde [1]	0.05 g Molybdic acid (or sodium molybdate) in 10 mL of hot conc. H_2SO_4(aq).	Heroin	Purple
Liebermann [2]	1.0 g $NaNO_2$ in 10 mL H_2SO_4(aq).	Acetaminophen	Violet
Mandelin[2]	0.5 g NH_4VO_3 dissolved in 1.5 mL water and dilute to 100 mL with H_2SO_4(aq). Filter through glass wool.	Procaine	Orange
Marquis[1]	Ten drops of 40% formaldehyde to 10 mL of conc. $H_2SO_{4(aq)}$ made as needed.	Heroin	Purple
Meckes[1]	0.25 g selenious acid in 25 mL conc. $H_2SO_{4(aq)}$.	Heroin	Green
Ruyball test[3]	Mix 9 parts of 2% (w/v) sol'n of $Co(SCN)_2$ in water and 3 parts H_3PO_4, add 1 part 1 g H_2PtCl_6, $6H_2O$ in 20 mL of H_3PO_4 and mix well. Add 9 parts water. Mix and let stand for 5 to 7 days.	Cocaine base	Blue
Van Urk[4]	Dissolve 1.0 g of *p*-dimethylaminobenzaldehyde (*p*-DMAB) to 25 mL of 95% EtOH and 50 mL of conc. HCl.	LSD	Violet

Procedure for tests in Table 1.

- Add 1-2 drops of reagent to a well within the well plate. As long as the reagent in the well plate is consistent with the appearance of the reagent in bulk, this serves as the blank and will be documented in the notes.
- Add approximately 1 mg of sample to the reagent and stir if necessary. Observe and record physical changes such as color change and effervescence.

40.4.2 TABLE 2. TWO STEP COLOR SCREENING TESTS

Test Name	Reagent Formula	Positive Control	Expected Result
Dille-Kopanyi[4]	Sol'n 1: Add 0.1 g $Co(OAc)_2$ to 100 mL MeOH, then add 0.2 mL glacial $HOAc_{(aq)}$ and mix. Sol'n 2: Add 5 mL isopropylamine to 95 mL MeOH.	Barbiturate	Light purple
Simon's (modified sodium nitroprusside test)[4]	Sol'n 1: Dissolve 1 g of sodium nitroprusside in 100 m: of water, add 2 mL of acetaldehyde to the sol'n with thorough mixing. Sol'n 2: Freshly prepared 2% sodium carbonate in distilled water.	Methamphetamine	Dark Blue

Weber[5]	Sol,n 1: 0.1g Fast Blue B or Diazo Bule B (o-dianisidine, tetrazotizod) in 10 mL water – must be made daily. Sol’n 2: Conc. HCl	Psilocyn	Red, Blue

Procedure for tests in Table 2.

- Add 1-2 drops of first reagent to a well in a spot plate. Add 1-2 drops of the second reagent. This serves as a blank and is documented in the case notes.
- Add 1-2 drops of first reagent to a well in a spot plate. Add approximately 1 mg of sample to the reagent and stir if necessary to dissolve. Add 1-2 drops of the second reagent. Observe and record physical changes such as color changes and effervescence.

40.4.3 OTHER COLOR SCREENING TESTS

Scott test[4]

- Solution 1: 2% Co(SCN)2(aq) diluted 1:1 with 96% glycerin.
- Also necessary: Conc. HCl(aq) and chloroform.
- Test material: Cocaine
- Expected result: See below
- Procedure
  - Place a small amount of the suspected cocaine in a test tube. Add five drops of Solution 1 and shake. If cocaine is present a blue color develops at once. If a blue color does not develop, the sample may not contain cocaine.
  - Add 1 drop conc. HCl(aq) and shake. The blue will disappear and a clear pink solution is seen. If all the blue does not disappear, add a second drop (no more) of conc. HCl(aq) and shake.
  - Add several drops of chloroform and shake. The bottom layer will develop an intense blue color if cocaine is present.
  - Observe and record color changes after each step for the sample and blank.

Chen’s test[6]

- Chemicals: Acetic Acid, copper sulfate and sodium hydroxide.
- Test Material: Ephedrine.
- Expected result: Purple-blue.
- Procedure
  - Place a small amount of the suspected ephedrine in 0.5 mL water. If the material is slow to dissolve, add a drop of acetic acid.
  - Add one drop of 10% CuSO4(aq), then 0.5 mL 20% NaOH(aq) solution and mix thoroughly. A purplish color will appear if ephedrine is present.
  - Add 0.5 mL ether – color should extract into the ether layer.
  - Observe and record color changes in the case notes for the sample and blank.

40.4.4 REFERENCES

1. Butler WP. Methods of Analysis for Alkaloids, Opiates, Marihuana, Barbiturates, and Miscellaneous Drugs. Internal Revenue Service (Reprinted by the Bureau of Narcotics and Dangerous Drugs, U.S. Department of Justice), rev. 6-67.
2. Moffat AC. 1986. Clarke’s Isolation and Identification of Drugs, 2nd Ed. London: Pharmaceutical Press.
3. (a) Ruybal R. 1972. Microgram. 5(3). (b) Ruybal C. Microgram. 6(2).
4. Moffat AC, Osselton MD, Widdop B. eds.2004. Clarke’s Analysis of Drugs and Poisons, 3rd Ed. London:Pharmaceutical Press.
5. Garrett AS, Siemens SR, Gaskill JH. 1992. The Weber Test: A Color Test for the Presence of Psilocin in Mushrooms. NWAFS Journal. 18(4).

6. Chen KK, Kao CH. 1926. Ephedrine and Pseudoephedrine, their isolation, constitution, isomerism, properties, derivatives, and synthesis. J. Am. Pharm. Assoc. 15(8):625-639.

40.5 COMMON SEIZED DRUG THIN LAYER CHROMATOGRAPHY (TLC) VISUALIZING AGENTS

The following are TLC visualizing agents commonly employed in the analysis of seized drugs. Many other visualizing agents are well documented in literature and the following list is not intended to be all inclusive. The superscript number listed with the test name indicates the reference describing the visualizing agent.

Visualizing Agent	Formulation and Procedural Notes
Ceric sulfate [2]	5g $Ce(SO_4)_2$ in 500 mL water and 14 mL sulfuric acid. Used as an overspray to intensify the reaction with iodoplatinate.
Dragendorff [1]	1.3 g of bismuth subnitrate in 60 mL water with 15mL acetic acid. Add this to 12 g KI in 30 mL water. Dilute with 100 mL of water and 25 mL acetic acid. General spray, good for diazepam, alkaloids and nitrogenous compounds.
Ehrlich's (*p*-DMAB) [1]	2g p-dimethylaminobenzaldehyde in 50mL 95% ethanol and 50 mL concentrated HCl. Visualizes LSD, reacts with indole nucleus of alkaloids. Heat plate to intensify color.
Fast Blue B [1,]	Approx. 0.5% to 1% solution of Fast Blue B in water. Used for marihuana. Δ9-THC — red, cannabidiol — orange, cannabinol — purple
Fluorescamine [1]	25 mg in 100 mL acetone. Visualize amino acids, amines and amino sugars. Heat after spraying, check under long wavelength UV light.
Furfuraldehyde [1]	10% Furfuraldehyde in ethanol, HCl. May heat plate after spraying. For non-aromatic carbamates; black spots.
HCl - 6N	Used to acidify plates (e.g., with Fast Blue B for psilocyn/psilocybin).
Iodoplatinate, Acidified [1]	0.25 g platinic chloride and 5 g potassium iodide in water to produce 100 mL. Add 5 mL of HCl to the 100 mL iodoplatinate solution. Used for nitrogenous compounds.
Mercuric chloride-Diphenylcarbazone [1]	Solution A: 0.1 g diphenylcarbazone in 50 mL ethanol; Solution B: 1 g mercuric chloride in 50 mL ethanol. Mix solutions just before spraying. Used for barbiturates.
Ninhydrin [1]	Ninhydrin in various solvents. For amino acids, amines and amine sugars. Heat after spraying, view under long wave UV.
Potassium Permanganate, acidified [1]	1% potassium permanganate in 0.25 M sulfuric acid. Unsaturated hydrocarbons — yellow on purple.
Sulfuric Acid/Ethanol [1]	Gradually add 10mL conc. sulfuric acid to 90mL of ethanol. Used for steroids.

40.5.1 REFERENCES

1. Hamilton R. Hamilton S. 1978. Thin Layer Chromatography: Analytical Chemistry by Open Learning. London:Johan Wiley and Sons.
2. Moffat AC, Jackson JV, Moss MS, Widdop B. eds.1986. Clarke’s Analysis of Drugs and Poisons, 2nd Ed. London:Pharmaceutical Press.

41 APPENDIX B: FTC GENERIC NAMES AND DEFINITIONS FOR MANUFACTURED FIBERS

Federal Trade Commission Rules and Regulations Under the Textile Products Identification Act, 16 CFT Part 303

Acetate:	A manufactured fiber in which the fiber-forming substance is cellulose acetate. Where not less than 92% of the hydroxyl groups are acetylated the term triacetate may be used as a generic description of the fiber.
Acrylic:	A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer comprised of at least 85% by weight of acrylonitrile units.
Anidex:	A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer composed of at least 50% by weight of one or more esters of a monohydric alcohol and acrylic acid.
Aramid:	A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polyamide in which at least 85% of the amide linkages are attached directly to two aromatic rings.
Azlon:	A manufactured fiber in which the fiber-forming substance is composed of any regenerated naturally occurring proteins.
Elastoester:	A manufactured fiber in which the fiber-forming substance is a long-chain synthetic polymer composed of at least 50% by weight of aliphatic polyether and at least 35% by weight of polyester.
Fluoropolymer:	A manufactured fiber containing at least 95% of a long-chain polymer synthesized from aliphatic fluorocarbon monomers.
Glass:	A manufactured fiber in which the fiber-forming substance is glass.
Lyocell:	A manufactured fiber composed of precipitated cellulose and produced by a solvent extrusion process where no chemical intermediates are formed.
Melamine:	A manufactured fiber in which the fiber-forming substance is a synthetic polymer composed of at least 50% by weight of a cross-linked melamine polymer.
Metallic:	A manufactured fiber composed of metal, plastic-coated metal, metal-coated plastic, or a core completely covered by metal.
Modacrylic:	A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer composed of less than 85% but at least 35% by weight of acrylonitrile units.
Novoloid:	A manufactured fiber containing at least 85% by weight of a cross-linked novolac.
Nylon:	A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polyamide in which less than 85% of the amide linkages are attached directly to two aromatic rings.

Nytril: A manufactured fiber containing at least 85% of a long-chain polymer of vinylidene dinitrile where the vinylidene dinitrile content is no less than every other unit in the polymer chaing.

Olefin: A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer composed of at least 85% by weight of ethylene, propylene, or other olefin units.

PBI: A manufactured fiber in which the fiber-forming substance is a long-chain aromatic polymer having reoccurring imidazole groups as an integral part of the polymer chain.

PLA: A manufactured fiber in which the fiber-forming substance is composed of at least 85% by weight of lactic acid ester units derived from naturally occurring sugars.

Polyester: A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer composed of at least 85% by weight, of an ester of a substituted aromatic carboxylic acid, including but not restricted to substituted terephthalate units and parasubstituted hydroxybenzoate units. Where the fiber formed by the interaction of two or more chemically distinct polymers (of which none exceeds 85% by weight), and contains ester groups as the dominant functional unit (at least 85% by weight of the total polymer content of the fiber), are which, if stretched at least 100%, durably and rapidly reverts substantially to its unstretched length when the tension is removed, the term elasterell-p may be used as a generic description of the fiber.

Rayon: A manufactured fiber composed of regenerated cellulose, as well as manufactured fibers composed of regenerated cellulose in which substituents have replaced not more than 15% of the hydrogens of the hydroxyl groups. When the fiber is composed of cellulose precipitated from an organic solution in which no substitution of the hydroxyl groups takes place and no chemical intermediates are formed, the term lyocell may be used as a generic description of the fiber.

Rubber: A manufactured fiber in which the fiber-forming substance is comprised of natural or synthetic rubber, including the following categories:

(1) A manufactured fiber in which the fiber-forming substance is a hydrocarbon such as natural rubber, polyisoprene, polybutadiene, copolymers of dienes and hydrocarbons, or amorphous (noncrystalline) polyolefins.
(2) A manufactured fiber in which the fiber-forming substance is a copolymer of acrylonitrile and a diene (such as butadiene) composed of not more than 50% but at least 10% by weight of acrylonitrile units. The term lastrile may be used as a generic description for fibers falling within this category.
(3) A manufactured fiber in which the fiber-forming substance is a polychloroprene or a copolymer of chloroprene in which at least 35% by eight of the fiber-forming substance is composed of chloroprene units.

Saran: A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer composed of at least 80% by eight of vinylidene chloride units.

Spandex: A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer composed of at least 85% of a segmented polyurethane.

Sulfar: A manufactured fiber in which the fiber-forming substance is a long-chain synthetic polysulfide in which at least 85% of the sulfide linkages are attached directly to two aromatic rings.

Vinal: A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer composed of at least 50% by eight of vinyl alcohol units and in which the total of the vinyl alcohol units and any one or more of the various acetal units is at least 85% by weight of the fiber.

Vinyon: A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer composed of at least 85% by weight of vinyl chloride units.

42 APPENDIX C: FABRIC AND CORDAGE TERMINOLOGY

Braid: The intertwining (not twisting) of three or more strands to make a rope/cord.

Cordage: Any type of rope, string, twine, etc. made from twisting or braiding yarns together to produce a long strand either with a single ply or multiple plies.

Cord: A thin rope made of several strands braided or twisted together with an overall diameter less than 3/16”.

Core: Fibers, one or more filaments, or other material running lengthwise through the center of a rope or other cordage.

Course: The row of loops or stitches running across a knit fabric, corresponding to the weft in woven fabrics.

Crown: The raised portion of a strand in twisted cordage.

Knit fabric: A structure produced by interloping one or more ends of yarn or comparable material.

Filament: A single continuous fiber extruded to an indefinite length.

Fusing: Uniting together as by melting together.

Nonwoven: Fabrics made directly from fibers held together by mechanical, chemical and/or thermal means.

Pitch: The number of crowns per inch along the length of the cordage.

Ply: One of the individual yarns that are twisted together to form a cord.

Rope: A heavy, strong cord made from natural or manufactured fibers with an overall diameter greater than 3/16”.

Selvage: The narrow edge of woven fabric that runs parallel to the warp. It is made with stronger yarns in a tighter construction than the body of the fabric to prevent unraveling.

Staple: Natural fibers or manufactured fibers cut into short lengths.

Strand: The largest individual element used in the final rope making process and obtained by joining and twisting several yarns or groups of yarns.

Thermoplastic: A synthetic material that is semi-permanently fusible or softens at high temperatures.

Thread: A slender, strong strand or cord made by plying or twisting yarns, typically sued for stitching.

Tracer: A yarn or yarns different in color, size and/or composition from that of the basic cordage found within or alongside a ply, strand or braid.

Twine: Two or more twisted strands or a single-strand yarn with an overall diameter less than 4 millimeters, made from natural fibers.

Twist, Direction of: The direction of twist in yarns is indicated by the capital letters S and Z. A yarn has an S-twist if, when it is held vertically, the spirals around its central axis slope in the same direction as the middle portion of the letter S, and Z-twist if they slope in the same direction as the middle portion of the letter Z.

Wale: A column of loops lying lengthwise in a knit fabric, corresponding to the warp in woven fabric.

Warp: The set of yarns in all woven fabrics that fun lengthwise and parallel to the selvage and is interwoven with the weft.

Weft (Filling): In a woven fabric, the yarn running from selvage to selvage at right angles to the warp.

Woven fabric: Generally used to refer to fabric that is formed by the perpendicular interlacing (weaving) of warp and weft (filling) yarns.

Yarn: A general term for a continuous strand of textile fibers, filaments or material in a form suitable for intertwining to form a textile structure via any one of a number of textile processes (e.g., knitting, weaving).

43 APPENDIX D: PRESSURE SENSITIVE TAPE TERMINOLOGY

Adhesive: A material that will hold two or more object together solely by intimate surface contact.

Additives: Materials that are included in adhesives or backing formulations to increase overall volume, impart color, or provide other desired properties.

Backing: A thin flexible material to which adhesive is applied.

Backsizing: A layer applied to the top side of the backing. Its purpose is to coat and fill a porous surfaced backing with a material that is inert to the adhesive formulation to be used.

Biaxially oriented polypropylene (BOPP): An oriented polypropylene film in which the polymer has been stretched in both the machine direction and cross direction during the manufacturing process. Tapes with such films cannot be torn by hand.

Calendering: The use of a multi-roll device to apply pressure sensitive adhesive at 100% solids to various backings by heat and pressure to produces adhesive tape.

Cellophane: A form of regenerated cellulose. A thin transparent film manufactured from wood pulp. Used as a backing material in tape products.

Cellulose acetate: A transparent film that is used for tape backings. A matte surface version is used for write-on tapes. It is more moisture-resistant that cellophane.

Creped: Paper that has small folds in it giving it high stretch and conformability. Used in masking tape (saturated paper tape).

Delustrant: An agent used to alter the light reflected from a fiber causing a dulling effect.

Duct tape: Fabric-reinforced tape used for general utility applications.

Elastomer: A material that can be deformed, but when the forces are removed will return to its original form. Serves as the base material for PSAs.

Electrical tape: Polyvinyl chloride (PVC)-backed tape with specific dielectric properties designed for electrical applications.

Filament tape: A fiber-reinforced tape in which the reinforcing fibers are only in the warp direction; also referred to as strapping tape.

Fill yarns: Fibers in the scrim fabric of reinforced tape that run cross-wise, perpendicular to the warp direction. Also called weft yarns.

Filler/extender: An inorganic material that is added to a tape to modify a physical property or reduce cost.

Flatback: Smooth paper backing sometimes used in masking tapes.

Long-wave UV Illumination: In the wavelength range from 400 nm – 315 nm with peak wavelength energy at 366 nm.

Machine direction: The direction of the tape that runs the length of the tape.

Masking tape: Paper-backed tape having a creped, usually beige or buff-colored backing. Painter's tape is a type of masking tape available in a number of colors.

Migration: The movement over a period of time of an ingredient from one layer to another. This often occurs in PVC tapes where plasticizer in the PVC backing "migrates" into the adhesive.

Monoaxially oriented polypropylene (MOPP): An oriented polypropylene film in which the polymer has been stretched in one direction during the manufacturing process. Tapes with such films can be torn by hand.

Monomer: A repeating structural unit within a polymer.

Nominal width: The design width of the tape, usually in terms of round numbers. Measured width can vary slightly from nominal width.

Packaging tape: a) Pressure-sensitive tape consisting of an oriented polymer with a brown or clear adhesive layer. b) Paper-backed tape, which has a moistenable adhesive.

Physical end match: A one-of-a-kind fit between two pieces of torn or cut ends demonstrating that the two pieces were once one continuous piece.

Plasticizer: Material added to plastics to impart flexibility by creating spaces between the polymer chains and lowering the inter- and intra-chain attractive forces, allowing freer movement of the chains. Used in pressure sensitive backings (particularly PVC) as well as some adhesives to lower glass-transition temperatures and allow use at sub-ambient temperatures.

Pressure Sensitive Adhesive (PSA): Consists of a polymeric base usually with appropriate plasticizers and tackifiers. It can form an adhesive bond with no physical or chemical change, and with no more than slight pressure.

Pressure Sensitive Tape (PST): Consists of a flexible backing and PSA, which when applied to a surface, bonds immediately at room temperature with slight pressure. The bond can be broken (usually) without damage to the surface and without leaving a residue.

Prime coat: A coating of adhesive-like material between the tape adhesive and backing that serves as a bonding agent.

Reinforcement: Cloth, scrim, glass filaments, or plastic filaments added to tape for stability and strength.

Release coat: A coating applied to the backing on the side opposite the adhesive that provides ease of unwind and prevents delamination or tearing.

Scrim: A loosely-woven gauze-type cloth added to duct tape for reinforcement and strength.

Scrim count: The dimensional count of the scrim, in terms of yarns per inch, expressed as warp count by fill count.

Short-wave UV illumination: In the wavelength range from 280 nm – 100 nm with the peak wavelength energy at 254 nm.

Strapping tape: See filament tape.

Tack: Property of an adhesive achieved by the addition of a low molecular weight organic component that allows the elastomer to form a bond immediately with a surface under low pressure.

Tackifier: Material added to the adhesive base polymer to impart tack.

Texturized yarn: Crimped reinforcement fibers designed to give bulk.

Thickness: Distance from one surface of either a tape, backing, or adhesive to the other, usually expressed in mils or thousandths of an inch.

Twist: The direction of twist in yarns is indicated by the capital letters S and Z. Yarn has an S-twist if when it is held vertically, the spirals around its central axis slope in the same direction as the middle portion of the letter S, and Z-twist if they slope in the same direction as the middle portion of the letter Z.

Warp yarns: Fibers in scrim fabric of reinforced tape that run lengthwise in the machine direction.

Weft yarns: See fill yarns.

Yarn: For the purposes of tape analysis, yarns refer to the lengths of fibers reinforcement: twisted staple fibers or filament fibers.

44 APPENDIX E: TAPE CONSTRUCTION AND CLASSES

44.1 BACKINGS:

The pressure sensitive tape backing, or film, provides a support material for the adhesive. There is a wide range of material used for tape backings depending upon the commercial end use. These include, but are not limited to, polyethylene, polypropylene, polyvinylchloride, saturated paper, cellulose acetate, cloth, and polyester. Furthermore, fillers, colorants, plasticizers, release coats, and preservatives may also be added to tape backings.

44.2 ADHESIVES:

The formulation of pressure sensitive adhesives (PSA) consists of an elastomer to which tackifier resins and inorganic materials are added.

44.3 ELASTOMERS:

The following is a list of elastomers that are sued in PSAs. PSAs may contain one elastomer or a blend of several different elastomers. Tackifying resins are blended with elastomer to lower the glass transition temperature, allowing freer movement of the polymer chains and this giving PSAs their "sticky" adhesive property. The tackifying resin is typically a C-5 (5 carbon hydrocarbon component). Silicone and acrylic PSAs do not require a tackifier. More costly silicone-based adhesives may be found in adhesive formulations of tape that are geared for high temperature or chemical resistance.

- Natural rubber (polyisoprene)
- Synthetic polyisoprene
- Polybutadiene
- Polyisobutylene
- Styrene butadiene random copolymer
- Styrene isoprene block copolymer (SIS)
- Styrene butadiene block copolymer (SBS)
- Styrene ethylene-butylene block copolymer
- Ethyl or butyl acrylate
- Silicones
- Polychloroprene

44.4 ADDITIVES:

Inorganic materials are added to an adhesive formulation to either increase the overall volume or to impart color. Such materials include calcite, dolomite, iron oxide, kaolinite, talc, titanium dioxide (rutile or anatase), and zincite. In addition, zincite can also function as an "accelerator," or cross-linker for a rubber-based adhesive. Other materials may be added to provide resistance to extremes in temperature or UV exposure.

44.5 TAPE CLASSES:

44.5.1 POLYCOATED CLOTH TAPE:

Commonly referred to as duct tape, polycoated cloth tape consists of three basic components: the backing, the reinforcement fabric, and the PSA. These components in concert are what determine a duct tape's appearance, strength, and end use. The final product will be designed for specific end usage, whether it is for general commodity use, construction, etc.

Duct tape backing:

The backing, which is polyethylene, is available in various colors. Duct tapes that are silver or gray commonly contain a small amount of aluminum to impart the silver color. Other colored backings are achieved by adding colored pellets to the molten polyethylene. Inorganic materials may be added to the backing, such as talc, which improves water repellency and tear strength. The backing may consist of a single layer or multiple layers of polyethylene and can range in thickness from about 1.5 mils to 4 mils (1 mil = 0.0010 in). The backing may also exhibit characteristics imparted during the manufacturing process, such as calendaring marks and striations. Additionally, lettering or design may also be imparted on the surface or the underside of the polyethylene.

Duct tape adhesive:

The PSA formulation for duct tapes consists of an elastomer to which tackifying resins and inorganic materials are added. The elastomer is typically natural rubber (polyisoprene) but could also be a mixture/blend of synthetic and/or natural elastomers. Other materials used as elastomers include styrene-butadiene copolymer and styrene-isoprene copolymer. The tackifying resin is typically a C-5 (5 carbon hydrocarbon component) that is used to make the elastomer "sticky" or impart tack.Inorganic materials are added to an adhesive formulation to either increase the overall volume or to impart color. In duct tape adhesives any of the following may be found: calcite, dolomite, kaolinite, talc, titanium dioxide and zincite.

Duct tape reinforcement fabric:

The scrim is commonly constructed of cotton, polyester, or a blend of these two materials. Reprocessed cellulose may also be found. The scrim is generally manufactured as either plain weave or weft-insertion (having knit warp yarns and texturized fill yarns). Yarns in both the warp and fill directions can be twisted (spun), texturized, or filament. Variations of these can be seen.

Other components found in duct tape:

Two additional layers that may be present within a duct tape product are a release coat and a primer coat.

44.5.2 VINYL TAPE:

A vinyl tape, also referred to as an electrical tape, finds use in applications that require heat resistance/retardance and insulating properties. The two main components are the backing and the PSA.

Vinyl tape backing:

Polyvinyl chloride (PVC) is the most common material used to construct the backing. Plasticizers, typically phthalate or adipate compounds, are added to this material to impart flexibility to the PVC. Other plasticizers may include alkyl/aryl phosphate compounds and dialkyl tin compounds. Backings range in thicknesses of 4.5-7.5 mils and are commonly black in color, imparted by the addition of carbon black. However, a variety of colored backings are produced and available. In addition to plasticizer, inorganic materials such as lead stearate, lead carbonate, antimony oxide, kaolinite, calcite, and titanium dioxide may also be found.

Vinyl tape adhesive:

The adhesive can be formulated in several ways, depending on the intended end use market, and can be either colorless or black, through the addition of carbon black. Commonly available vinyl tapes consist of acrylic-based PSA or highly cross-lined rubber-based PSA. The adhesive layer may also exhibit plasticizers, either intentionally added by the manufacturer or as a result of migration from the backing layer.

Other components in vinyl tapes:

As with duct tape, two additional layers, a release coat and a primer layer, may be used in vinyl tapes.

44.5.3 POLYPROPYLENE PACKAGING TAPE:

Polypropylene packaging tape has been designed as a general-purpose tape used to seal packages. The two main components are the polypropylene backing and the adhesive.

Polypropylene packaging tape backing:

Packaging tape backings are typically clear but also can be found in various shades of tan or brown. The polypropylene, which is in the isotactic form, can be subdivided into two distinct types based upon their tear resistant properties: monoaxially oriented polypropylene (MOPP) and biaxially oriented polypropylene (BOPP). A monoaxially oriented backing is formed into a thin film by stretching the polypropylene material as it is slowly cooled in one direction only (length-wise) prior to introducing it into the tape manufacturing process. A biaxially oriented backing is manufactured by stretching the film in two directions (length-wise and width-wise). There is a distinct end-use or consumer difference between MOPP and a BOPP tape: a MOPP tape is marketed as a “hand-tearable” tape, and BOPP tapes require a cutting tool such as a dispenser. Total tape thicknesses are on the order of 1.5-2.0 mil. The thickness of the film alone typically varies from 0.9 to 1.0 mil but can range from 0.8 to 2.0 mil.

Polypropylene packaging tape adhesive:

Packaging tape adhesives are typically clear but are available in shades of tan or brown. Generally, when the backing is colored, the adhesive will be clear and vice versa. While clear adhesives contain no inorganic material, the colored adhesive may contain inorganic material such as iron oxide and titanium dioxide. Adhesive formulations typically are isoprene-based, styrene-isoprene copolymer-based (SIS), or acrylic-based.

Other components found in polypropylene packaging tape:

Two additional layers that may be present within a packaging tape product are a release coat and a primer coat.

44.5.4 SATURATED PAPER TAPE:

“Masking tape” consists of a paper backing, a saturant, and an adhesive. This type of tape is used as a masking material for paint applications and other general-purpose applications.

Saturated paper tape backing:

The backing of a paper tape is either flatback or creped paper, which has been saturated with carboxylated butadiene styrene, acrylonitrile butadiene, or a similar material. The purpose of a saturant is to fill porous material and boost the strength of the backing. The paper alone typically exhibits weak internal and external strengths, and the saturant sills the voids between the paper fibers adding strength to the product and minimizing absorption of paint products.

Saturated paper tape adhesives:

The adhesive for saturated paper tapes typically is an isoprene-based PSA or a styrene-butadiene block copolymer, either of which may contain inorganic filler. Acrylic-based adhesives have been used as well, but for outdoor or “clean release” formulations. These adhesives for saturated tapes are formulated with less tack since strong adhesion to a surface is less desirable in masking applications. As with most tapes, if the product is designed to endure exposure to high heat or chemical reagents, the formulation will be cross-lined to provide the needed strength.

Other components found within saturated paint tape:

The backsize layer is applied to the side of the backing opposite of where the adhesive will be applied. The main purpose of this layer is to coat and fill the porous surface of the backing with a material that is inert to the adhesive formulation to be used. There are a variety of materials that can be used for this purpose, such as acrylic and polyvinyl acetate, and the material used will depend upon the adhesive formulation. In conjunction with the adhesive formulation, a primer coat may be present.

44.5.5 OTHER TAPES:

The previous sections have discussed the more common types of tapes encountered within forensic casework. There are numerous other types of tape that may also be found less frequently. These types include, but are not limited to, filament/strapping tape, cloth/medical tape, and office tape.

Filament/strapping tape:

Filament tapes are similar to packaging tapes in construction with the addition of reinforcement material. The backing for this type of tape is typically constructed of oriented polypropylene (low cost) or polyester (high cost). The reinforcement filaments can be glass, polyamide fibers, or polyester fibers running in the machine direction. Adhesives found on such tape products can be colored, dependent upon inorganic material content, or colorless. The elastomer can be either isoprene or styrene-isoprene block copolymer.

Cloth tape:

Cloth tapes are most frequently used for medical and athletic purposes. Common cloth materials include natural and synthetic woven fabrics (e.g., cotton, polyester). Traditionally, adhesives were natural rubber-based, but in recent history have been largely replaced by acrylic copolymers and other synthetic elastomers.

Office tape:

Office or stationery tape is comprised of a backing and a PSA. The most common tape backings include cellulose acetate, cellophane, and polypropylene and can range in appearance from clear glossy or matter to a translucent yellow. The PSA can be isoprene-based, acrylic-based, or styrene-isoprene copolymer-based. As mentioned in the previous tape discussions, a release coat and a primer layer may also be present.

45 APPENDIX F: MINIMUM ANALYSIS REQUIREMENTS FOR IDENTIFICATION OF INTACT EXPLOSIVES

To report a specific brand, the highlighted compounds must be conclusively identified; all others must be indicated by one or more techniques.

Material Reported	Components Necessary for Identification
Black Powder	Potassium Nitrate Sulfur Carbon (Wood Charcoal)
Pyrodex® (Sulfur containing black powder substitute)	Potassium Nitrate Potassium Perchlorate Sodium Benzoate Dicyandiamide (Cyanoguanidine) Sulfur Carbon
Triple 7® (Sulfur-free black powder substitute)	Potassium Nitrate Potassium Perchlorate Sodium Benzoate 3-Nitrobenzoic Acid Dicyandiamide (Cyanoguanidine) Carbon *Absence of Sulfur*
Black Canyon®/Cleanshot® (Ascorbic acid based black powder substitute)	Potassium Nitrate Potassium Perchlorate Ascorbic Acid Carbon
Flash Powder	Metal Fuel Oxidizer Poss. Fillers/Binders
Smokeless Powder	Single Base: NC Double Base: NC, NG Triple Base: NC, NG, NGu Nitrate Esters *(ID as many as practical)* Stabilizers and Additives
Road Flare (red)	Strontium nitrate Potassium perchlorate Sulfur Sawdust Hydrocarbons
Smoke Signal/Smoke Bomb	Oxidizer Fuel Dye or colorant
Dynamite	Nitrate Esters *(ID as many as practical)* Nitroglycerin EGDN MTN DEGDN Ammonium/Sodium Nitrate Fillers/Binders Other Inorganic Salts

Material Reported	Components Necessary for Identification
Water Gels/Slurries	Inorganic Nitrate Gelling Agent (polysaccharides) Sensitizers *(ID as many as practical)* Microballoons Aluminum MMAN/EDDN
Emulsions	Inorganic Nitrate Oil/wax Emulsifiers Sensitizers *(ID as many as practical)* Microballoons Aluminum
ANFO	Ammonium Nitrate Hydrocarbon Mixture
Binaries (kinepak™, thermex™…)	Ammonium Nitrate Nitromethane
Plastic Bonded Explosives	Explosive (RDX, HMX, PETN) Dye Binder Plasticizer Oil DMNB (taggant)
Single Component Explosives TNT, PETN, RDX, HMX, EGDN, TATP, HMTD, Tetryl Primary Explosives: Azides, Styphnates, Fulminates, Diazodinitrophenol (DDNP)	ID the component

Abbreviations for Appendix F:

DDNP	Diazodinitrophenol
DEGDN	Diethylene glycol dinitrate
DMNB	2,3-dimethyl-2,3-dinitrobutane
EDDN	Ethylenediamine dinitrate
EGDN	Ethyleneglycol dinitrate
HMTD	Hexamethylene triperoxide diamine
HMX	Octagen; cyclotetramethylene tetranitramine
MMAN	Monomethylammonium nitrate
MTN	Metriol trinitrate (Trimethylolethane trinitrate)
NC	Nitrocellulose
NG	Nitroglycerin
NGu	Nitroguanidine
PETN	Pentaerythritol tetranitrate
RDX	Cyclonite; trimethylene trinitramine; hexahydro-1,3,5-trinitro-s-triazine
TATP	Triacetone triperoxide
Tetryl	Trinitro-2,4,6-phenylnitramine
TNT	2,4,6-trinitrotoluene

46 APPENDIX G: EXPLOSIVES AND THEIR POST-BLAST RESIDUES

This list was adapted from the Technical Working Group for Fire and Explosions Analysis (TWGFEX) Recommended Guidelines.

EXPLOSIVE	Common Post-Blast/Burn Particles & Unreacted Particles
Black Powder	NO_3^-, SO_4^{2-}, NO_2^-, CO_3^{2-}, HCO_3^-, SCN^-, OCN^-, S^{2-}, K^+, $S_2O_3^{2-}$
Pyrodex® (sulfur containing black powder substitute)	NO_3^-, SO_4^{2-}, CO_3^{2-}, Cl^-, HCO_3^-, ClO_4^-, benzoate, ClO_3^-, DCDA(CG), OCN^-, NO_2^-, K^+, SCN^-
Triple Seven® (sulfur-free black powder substitute)	NO_3^-,CO_3^{2-}, Cl^-, K^+, NO_2^-, ClO_4^-, ClO_3^-, OCN^-, HCO_3^-, nitrobenzoic acid, DCDA(CG), benzoate
Ascorbic Acid Based Black Powder Substitutes: e.g., Black Canyon®/Clean Shot®/ Pioneer®/Golden Powder®	Ascorbic acid, NO_3^-, CO_3^-, NO_2^-, K^+, ClO_4^-, ClO_3^-
Pyrotechnic formulations: e.g., flash powder	ClO_4^-, ClO_3^-, NO_3^-, metals, oxides, Ba^{+2}, Sr^{+2} , Al, K^+, Na^+, additives, dyes
Smokeless (single base)	Unreacted particles, NC
Smokeless (double base)	Unreacted particles, NC, NG
Smokeless (triple base)	Unreacted particles, NC, NG, NGu
Dynamite	Unreacted material, NG, EGDN, AN/SN (or ions), DEGDN, DNT
PETN, RDX, HMX, EGDN, HMTD, Tetryl	Parent compound found
TNT	TNT and often DNT's
Water gels and slurries	NO_3^-, MMA^+, NH_4^+
Emulsions	NO_3^-, NH_4^+
ANFO	NO_3^-, NH_4^+, NO_2^-, hydrocarbon mixture
Binaries	NH_4^+, NO_3^-
PBX	Unreacted materials
Primary explosives	Unlikely to survive, possible residual elements (e.g., lead, mercury)
Improvised Explosives/Incendiaries	
TATP	Peracetic acid, acetone
HMTD	Hexamine
Dry ice & water in bottle	No chemical evidence
Acetylene/natural gas/propane (often in a plastic bag)	Container fragments, fuse remains, odor?
Pool chlorine [Ca Hypochlorite] & and alcohol	Cl_2 odor, unreacted material, chlorinated products
Pool chlorine & brake fluid	Cl_2 odor, unreacted material, chlorinated products

EXPLOSIVE	Common Post-Blast/Burn Particles & Unreacted Particles
Pool chlorine & glycerin	Cl_2 odor, $CaCl_2{\approx}6H_2O$ and $CaCO_3$
Pool chlorine & milk products	Cl_2 odor, unreacted material, chlorinated products
Pool chlorine & oil (drying oils, vegetable oils, etc.)	Cl_2 odor, $CaCl_2{\approx}6H_2O$, $CaCO_3$, $CaCl_2$, $Ca(OCl)_2$, $Ca(OH)_2{\approx}2H_2O$, butyric acid?
Pool chlorine & antifreeze	Cl_2 odor, unreacted material, chlorinated products
Pool chlorine (organic) & alcohol	Cl_2 odor, unreacted material, chlorinated products
ANTI (NI_3) (Nit4I and Nitrogen triiodide)	Find NH_4I and yellow stain (I_2), spattering of unreacted material
Chlorate & sugar	KClO, NaCl, residual sugar, odor of burnt sugar, ClO_3^-
Paper match heads (safety matches)	KCl, SiO_2, paper fibers, unreacted match heads, S, SO_4^{2-}, S_2^-
Strike anywhere match heads	Unreacted match heads, P, S, SO_4^{2-}, S_2^-
Red phosphorous & chlorate (Armstrong's mixture)	P, ClO_3^-, K^+, Cl^-, PO_4^{3-}
HCl and aluminum	$Al_2Cl_6{\cdot}6H_2O$ and H_2O, unconsumed Al, acidic
HotPack or ActionPack	Magnesium hydroxide
Tannerite	Unreacted materials, NO_2^-, NO_3^-, NH_4^+, Al, ClO_4^-, Cl^-
Mischmetal	Sparks and unreacted material
Urea nitrate	Unreacted material, NO_2^-, NO_3^-, NH_4^+, urea
Al and I_2	Unreacted materials, brown/purple residue
50:50 Zn:S (both powders)	Zn, S, Zn^+, S^+
CCl_4 + Al powder (1:4)	Al
Vaseline and $KClO_4$ (1:2) (poor man's C-4)	ClO_4^-, unreacted material
$KMnO_4$ + glycerin	Unreacted materials, K^+, manganese salts
Drano + aluminum	Al, Na, basic pH
Al + lye/drano + water	$Al(OH)_3$, NaOH, basic pH, Al
Al + HCl	Al, Al^+, Cl^-, acidic

Abbreviations for Appendix G:

AN	Ammonium nitrate
ANFO	Ammonium nitrate fuel oil
CG	Cyanoguanidine
DCDA	Dicyandiamide
DEGDN	Diethylene glycol dinitrate
DNT	Dinitrotoluene (unspecified isomer)
EGDN	Ethyleneglycol dinitrate
HMTD	Hexamethylene triperoxide diamine
HMX	Octagen; cyclotetramethylene tetranitramine
MMA	Monomethylamine

Abbreviations for Appendix G:

NC	Nitrocellulose
NG	Nitroglycerin
NGu	Nitroguanidine
PBX	Plastic bonded explosive
PETN	Pentaerythritol tetranitrate
RDX	Cyclonite; trimethylene trinitramine; hexahydro-1,3,5-trinitro-s-triazine
SN	Sodium nitrate
TATP	Triacetone triperoxide
Tetryl	Trinitro-2,4,6-phenylnitramine
TNT	2,4,6-trinitrotoluene

47 APPENDIX H: GUIDELINES FOR REPORTING CONCLUSIONS OF MANUFACTURED MATERIALS

The process of arriving at a conclusion when conducting a comparative examination of manufactured materials generally follows a logical progression: 1) assessment of the submitted evidence and evaluation of possible limitations, 2) determination of the type(s) of materials present in the evidence, 3) selection of the appropriate analytical approach for comparison of the manufactured materials, 4) collection and evaluation of observations and analytical data, and finally 5) the formulation of conclusions/opinions/interpretations based on the comparative examination. The significance of that conclusion is based on the entire process, which should be communicated in the case notes and wording of the report.

Assessment of the submitted evidence needs to take into account any limitations such as sample size, physical condition of the sample, impact from the method(s) used to collect/recover the evidence, physical and/or chemical damage that may have occurred during or after the crime was committed, or other factors. It should also be understood that these evidential limitations may differ between Questioned samples and Known samples. These limitations and how they may affect the comparative examination are to be detailed and/or explained in the bench notes and report where appropriate.

Determination of the type(s) of materials present in an item of evidence involves assigning the materials(s), according to its chemical and physical properties (e.g. chemical composition, color, size, shape, and artifacts) into a category, or class, of similar items (e.g. paint, shoes, pencils, etc.). The characteristics that define that category are termed “class characteristics.” Class characteristics may change over time as manufacturing processes change. The variation of class characteristics within some of the manufactured materials that may be encountered as evidence is documented in the scientific literature. The variation inherent in less commonly encountered materials may be unknown or only partially documented in the literature. When documents are available that indicate the relevant characteristics of a material, a copy of that document may be included in the case file to support the analyst’s decision on case approach. Such documents may include limited published scientific data, shared unpublished scientific data, or materials provided by manufacturers.

Selection of the appropriate scheme for conducting examinations of manufactured materials will be determined based on known properties of the materials with various class characteristics, any limitations on the material, and the distinguishing capabilities of the available techniques. The techniques used may include any combination of visual or microscopic observations, microchemical testing and/or other non-instrumental approaches, and data collected from analytical instrumentation. Consultation with scientific literature and other experts may be appropriate. A limited survey of reference materials conducted by the analyst may be considered for less commonly encountered materials.

Data collected from all of the observations and analytical techniques used on both the Questioned and Known items are then compared to determine if a meaningful relationship between them can be made. The data should be sufficient to provide physical and chemical class characteristics that adequately demonstrate the details of exclusion or inclusion (including any unique or individualizing characteristics) that may be in the materials examined.

Conclusions can be formed once sufficient and appropriate data have been collected, documented, and compared. While an accepted statistical assessment of significance for such comparisons is not available, a non-statistical significance of the comparison can be made based upon the differential value of the examined characteristics and any unexplained dissimilarities. The analyst should be aware of published studies for comparison of commonly encountered materials. Information the analyst may have regarding the relative value of less commonly encountered materials should be noted in the case file. Such information may include limited published data, shared unpublished data, or a limited survey of reference materials conducted by the analyst.

A range of conclusions may not address every variable in every examination. Wording expressing conclusions in each case should be constructed specific to the results of the examination in that case. Observed characteristics used by the analyst to distinguish between one category or another should be stated in the notes. If additional analyses may provide probative information, such analyses should be communicated in the report. The accepted range of conclusions for the comparison of manufactured materials is outlined below:

Physical Match
Definition: The items that were compared conclusively physically fit back together with at least one individualizing characteristic shared across the fracture line.

Conclusion: The items were at one time part of the same object.

Examples: Broken pieces of manufactured materials (vehicle parts, glass, paint)

Similar with Atypical Characteristics
Definition: The materials that were compared could not be differentiated based on their physical and chemical properties. Furthermore, the materials share at least one characteristic that is not typically observed in this type of material.

Conclusion: The compared materials could have originated from the same source. Although another source of similar manufacturing cannot be ruled out, the shared atypical characteristic makes another source of similar manufacturing improbable.

Examples: After market repaints, materials with manufacturing defects, materials with characteristics acquired after manufacturing

Similar with Typical Characteristics
Definition: The materials that were compared could not be differentiated based on physical or chemical properties. The characteristics found in this examination are typically observed for this type of material.

Conclusion: The compared materials could have originated from the same source, or another source of similar manufacturing.

Examples: Fibers, Original Equipment Manufacturer (OEM) paint, tape, glass

Similar with Limited Characteristics

Definition: The materials that were compared could not be differentiated based on physical and chemical properties. Some of the typically observed characteristics of this material are absent, the sample size limits the number and/or types of examinations, the discriminatory value of some of the characteristics is unknown, or the characteristics are so common as to have been shown to have limited discriminatory value.

Conclusion: The compared materials could have originated from the same source, or another source of similar manufacturing. However, other sources for this type of material are common, and thus the conclusion has undetermined or limited value.

Examples: Questioned sample is a tape adhesive only (no backing), question sample is a single layer clear coat automotive paint, blue cotton fibers

Elimination/Exclusion

Definition: The items that were compared do not physically fit back together or the materials that were compared are physically or chemically different.

Conclusion: The items were not from the same object or the compared materials could not have originated from the same source as represented by the sample(s) provided.

Examples: Manufactured materials in general (fibers, glass, paint, tape, etc.) that do not physically fit or that do not share class characteristics (a questioned sample being polyester and the known being nylon)

Inconclusive/Unsuitable

Definition: The physical and chemical properties do not provide enough information to associate or eliminate the compared materials. The lack of information may be due to the constraints of sample size, the condition of the evidence, the limitations of the tests, or the results of the tests.

Conclusion: It could not be determined whether or not the compared materials originated from the same source as represented by the sample(s) provided. Sample and/or test limitations prevent a definitive association or exclusion.

Examples: Manufactured materials in general (fibers, glass, paint, tape, etc.)